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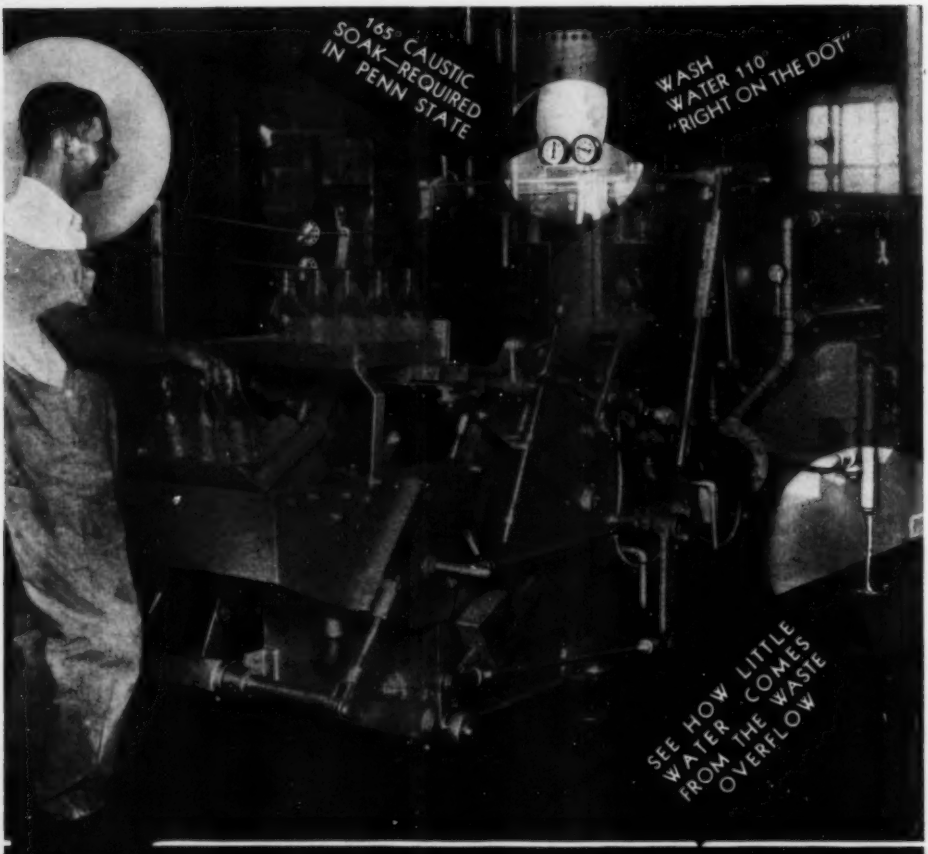
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JOURNAL OF DAIRY SCIENCE

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INVESTIGATION OF RESAZURIN AS AN INDICATOR OF THE SANITARY CONDITION OF MILK

GUY A. RAMSDELL, WM. T. JOHNSON, JR., AND F. R. EVANS

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U. S. Department of Agriculture

The use of chemical indicators for determining the sanitary condition of milk is finding considerable application. It is the purpose of this paper to describe our studies of the indicator resazurin. On the basis of the results of this investigation extending over two years, it is felt that more information as to the sanitary condition of milk can be obtained by the use of resazurin than from any other chemical indicator now in use.

While methylene blue fulfills quite well all the requirements of a reductase test as specified by Thornton and Hastings (1), it still falls far short of giving a true picture of the sanitary condition of many milk samples. Its time of reduction is dependent, in part, upon the rapidity of growth of those organisms that develop under a definite temperature of incubation and not upon the actual number of bacteria initially present,—that is, the original contamination. Also, it fails to classify properly those milks containing organisms that are slow reducers, since a population well into the millions must be developed before the Eh of the milk is sufficiently low to effect complete decolorization. Differences in reducing intensities of different organisms have been observed by Clark and his associates (2) and by Frazier and Whittier (3). Another objection is that the test takes too long. Thornton and Hastings (1) state that any oxidation-reduction indicator more electro-positive than methylene blue will give misleading results. Clark (2), on the other hand, has expressed the belief that a more electro-positive indicator might be advantageous. Our results, with the slightly more electro-positive indicator resazurin, bear out Clark's opinion. This is especially true if the sanitary condition of milk is to include the pathological and physiological abnormalities as well as the bacterial contamination.

Resazurin as an indicator of the sanitary condition of milk has received some attention abroad, but there has been no reference in the literature to its use for this purpose in this country. Comparisons of reduction time of resazurin and bacterial numbers were made by Olga Waldbauer (4).

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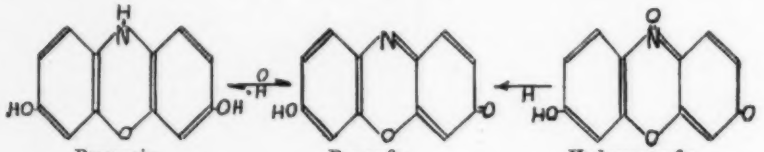
This author, on the basis of her results, was able to classify milks into four groups. Mundiger and Wolf (5) compared the reduction time of resazurin with plate and Breed counts. They conclude that claim cannot be laid to an accurate bacterial count by the resazurin test, but that the test is a good means of approximating the quality of milk, is applicable to control work, and is especially useful for rapidly detecting poor milk of high bacterial numbers. These authors state that resazurin in milk reduces to resorufin, which, in turn, reduces to the colorless modification hydroresorufin.[1] These steps are shown below.



The reduction of resazurin in milk is not reversible, while the change of resorufin to hydroresorufin is reversible. Resazurin in milk at a pH of 6.5 is blue and at a pH of 5.3 is red, while resorufin is pink at a pH of 6.5 and yellow at a pH of 4.8. A mixture of resazurin and milk that is becoming progressively more negative in Eh will change from blue to pink through various shades of purple and lavender. The dye has a high tinctorial power and the color changes are easily judged.

EXPERIMENTAL

It was found that an aqueous solution of resazurin containing .05 per cent of the dye did not undergo any visible decomposition when confined in the dark for six months. Consequently, a solution of this concentration was used for stock solution, and when 0.1 ml. was added to 10 ml. of milk the color produced was sufficient for good tinctorial effect. The germicidal effect of this concentration, *i.e.*, one part of dye to 200,000 parts of skim milk, was then determined. The results are given in Table 1. It is observed that resazurin does produce a significant toxic effect in milk, but methylene blue in concentrations of one to 571,000 suppressed growth practically to the same extent. However, this toxicity does not seriously operate against the use of resazurin as a reduction test for sanitary condition. In fact, the stock solution is not germicidal for all organisms, hence considerable care must be exercised to keep it sterile.

[1] We have been unable to obtain the same shade of pink as developed when resazurin-milk mixtures are incubated by adding the chemical resorufin to milk in like proportion. This seems to throw some doubt upon whether the difference is due to the resorufin used or whether resazurin actually changes to resorufin during the course of reduction. These authors state that their dye contained pure resazurin together with a small percentage of resorufin.

TABLE 1

Germicidal effect of resazurin and methylene blue in skimmed milk

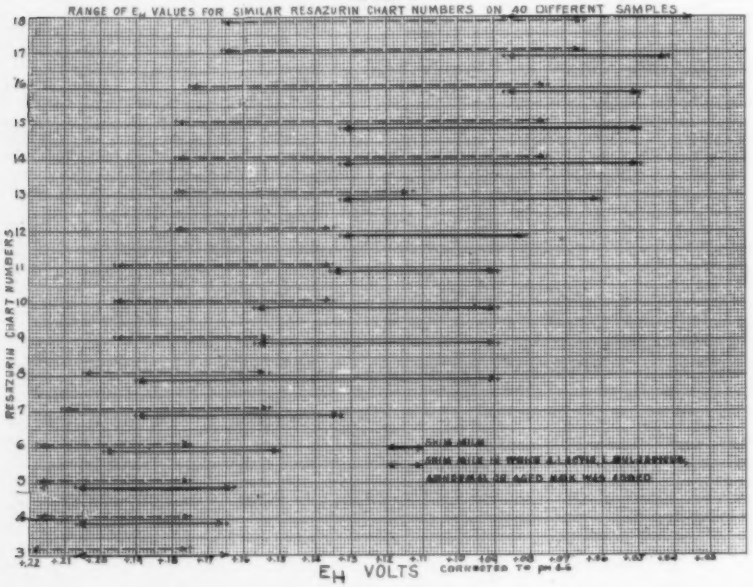
SERIES NO.	HOURS INC. AT 37° C.	MICROSCOPIC COUNT IN MILLIONS				% REDUCTION IN MULTIPLICATION BY ADDIT. OF IND.		
		CONTROL	Resaz-Skim. 1 : 200,000	Resaz-Skim. 1 : 400,000	MB-Skim. 1 : 571,000	Resaz-Skim. 1 : 200,000	Resaz-Skim. 1 : 400,000	MB-Skim. 1 : 571,000
1	0	2.6	2.8	.....	2.8	.....	.....	.....
"	3	110.0	78.0	.....	76.0	30.0	.....	31.9
2	0	2.9	3.3	2.8	3.2	.....	.....	.....
"	3	130.0	100.0	110.0	110.0	22.9	15.6	15.9
3	0	2.5	2.4	2.4	2.2	.....	.....	.....
"	3	63.0	59.0	60.0	52.0	3.6	2.7	9.0
4	0	1.6	1.8	1.6	1.7	.....	.....	.....
"	3	84.0	56.0	70.0	58.0	33.5	17.0	31.7
"	5	800.0	660.0	640.0	680.0	17.6	20.0	15.0
5	0	3.6	3.0	3.6	3.5	.....	.....	.....
"	3	78.0	59.0	80.0	64.0	24.7	0	18.7
"	5	700.0	500.0	500.0	450.0	28.6	28.7	35.9
6	0	.1	.1	.....	.....	.....		
"	3½	12.5	7.6	.....	.....	39.5		
"	4½	13.6	9.4	.....	.....	31.1		
"	5	27.0	17.0	.....	.....	37.2		
7	0	.096	.08	.....	.....	.....		
"	3½	1.0	1.6	.....	.....	40.5*		
"	5½	40.0	28.0	.....	.....	30.0		

* Increase.

To identify the various colors developed in resazurin-milk mixtures during incubation, comparisons were made with a color chart. The chart contained 10 different color standards, each color being given an odd number in increasing sequence to its diminishing proportion of blue, so that the last standard was numbered 19 and was entirely pink. Standard No. 1 corresponded to the color produced by adding resazurin to fresh skim milk in concentration of one of dye to 200,000 of milk. While the number of standards used in the chart was chosen arbitrarily, and possibly is greater than would be considered necessary, especially by one running a large number of tests daily, it was found that there was sufficient variation in color between any two consecutive standards to be easily distinguished. Likewise, the more standards, the more accurate would be the determination of the amount of reduction. The standards were prepared by filling 20 ml. test tubes with mixtures of various proportions of undyed china clay and china clay dyed with brom cresol green, crystal violet, and alkaline phenolphthalein.

Comparisons were made between Eh values measured potentiometrically during the potential drift and the developed colors on 40 different samples of resazurin-skim milk mixtures. There was quite a spread in Eh values corresponding to a single resazurin number, so that it was impossible to interpret intensities on the basis of the developed color. The maximum and minimum Eh values obtained for each resazurin number on this series of samples are presented in Figure No. 1. Eh measurements were not

FIG. NO. 1



obtained for all of these extremes, this procedure being impracticable; consequently, they are not entirely valid for each color number. However, the variations between the true and plotted values should not vary by over .005 volt. The zone of reduction of resazurin to resorufin as determined visually by the disappearance of the blue modification in 26 samples of skim milk in which no abnormal milk or special cultures had been added was never more positive than +.10 volt, or more negative than 0 volt. The zone of decolorization of resorufin of these samples fell within the Eh range of +.05 and −.05 volt. In market milk the zone of reduction of these two systems is approximately .1 to .05 volt more positive. In all the physiologically abnormal and pathological milks[2] tested the zones of reduction were shifted to even more positive values.

[2] Throughout this paper the term "physiologically abnormal milks" refers to colostrum and milk from cows drying up, and "pathological milks" refers to milk from cows with diseased udders.

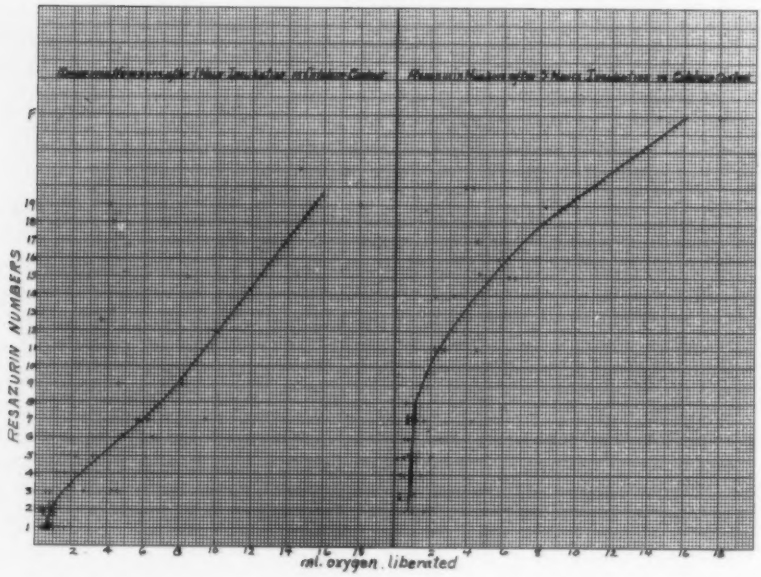
If an oxidation-reduction indicator were used to measure only bacterial contamination, then even methylene blue, which in some milks decolorizes at Eh levels as positive as the upper limits of milk systems (1), *i.e.*, from +.2 to +.3 volt, would give misleading results, and obviously indicators more electro-positive would be even less dependable. However, leucocytes or substances associated with leucocytes reduce methylene blue and if they do not produce a corresponding lowering in oxygen level, then their presence might account, to some extent at least, for the shifting of the zone of methylene blue or resazurin reduction to more positive potentials. Consequently, if this shift is produced by substances present only in physiologically abnormal and pathological milks, then it seems that an indicator such as resazurin, which is reduced by these abnormal milks, and, being slightly more electro-positive than methylene blue but more electro-negative than normal, fresh milk, would respond more rapidly to the reducing influences of abnormal milks and thus, instead of yielding misleading results, be of added value as an indicator of quality over that of methylene blue. Also, with a dye such as resazurin which reduces irreversibly to another colored compound, any slight reduction would be manifest in an alteration of color and thus yield a measurable record of any abnormality or of any slight potential drift.

COMPARISON OF RESAZURIN REDUCTION AND VARIOUS TESTS USED TO DETECT ABNORMAL CONDITIONS OF MILK

In order to learn to what extent resazurin is reduced by physiologically abnormal and pathological milks, comparisons were made between the rate of reduction of resazurin and tests used to detect abnormal milks. Middle milk was used and was obtained from individual quarters. So as to eliminate so far as possible the reducing effect of fortuitous bacterial contamination, samples were drawn directly into sterile bottles. The milk was morning's milk and only a few hours had elapsed from the time of milking until the tests were made. The tests included analyses for catalase and chlorides, cell counts, microscopic counts before and after five hours incubation at 37° C., and standard agar plate counts, together with methylene blue and resazurin reduction tests. The course of reduction of the resazurin samples was determined by comparison with the color chart every hour for five consecutive hours. It was observed that in every sample of high resazurin number, *i.e.*, 11 or over within the first two hours, there was also a high cell count of one million or more, regardless of the bacterial analysis.

The relationship between resazurin reduction and catalase content of these 49 samples is shown in Figure no. 2. In the samples of low catalase and small bacterial population only a slight resazurin reduction resulted even after three hours of incubation. However, a high catalase content was invariably associated with considerable reduction of this indicator.

FIG. NO. 2



On the other hand, where the reduction of resazurin was high without a correspondingly high catalase content, the divergence resulted from the fact that these milks were of high bacterial numbers. Methylene blue decolorized in less than eight hours in only two of the 49 samples. One sample containing 16 million cells did not fade methylene blue before eight hours, but the resazurin number after only one hour of incubation was 15, indicating considerable reduction.

While it is still a mooted question whether high leucocyte content in milk is deleterious, especially if the leucocytes do not come from suppurative discharges, the mere existence of large numbers is certainly indicative of the fact that the milk is abnormal. And since it has been shown by Rosell (6) that mastitis milk is quite prevalent, a test that responds to this type of milk as well as to bacterial contamination is of additional value as a test of quality over that with methylene blue.

COMPARISON BETWEEN METHYLENE BLUE AND RESAZURIN TESTS

Comparison between methylene blue and resazurin tests were made on 220 market milk samples. The methylene blue solution was prepared by dissolving one methylene blue tablet in 200 ml. of sterile water. One ml. of this solution was added to 10 ml. of milk. The resazurin numbers were obtained by comparing the developed color with the color chart after the

resazurin-milk mixtures containing one of dye to 200,000 of milk were incubated for one hour at 37° C. From the results obtained the milks were graded into four classes according to the following scheme:

*Methylene Blue Reduction Time	Class	Resazurin Number After One Hour Incubation
More than 5½ hours	1	1 to 6
2 to 5½ hours	2	7 to 18
20 mins. to 2 hours	3	19 to nearly faded
Less than 20 minutes	4	Completely faded

Comparison of 410 additional market milk samples were made. In this latter series the methylene blue concentration in milk was in the ratio of one to 200,000. This methylene blue-milk mixture contained 2.85 times more methylene blue than that used in the standard method where methylene blue tablets were used. In this latter series Class 1 with the resazurin test included all colors from 1 to 7, Class 2 numbers 8 to 18, and the remaining two classes similar to the former classification. The results of these two series of comparisons are given in Table 2.

TABLE 2

Comparison between methylene blue and resazurin tests

CLASSIFICATION BY RESAZURIN TEST	CLASSIFICATION BY METHYLENE BLUE TEST CLASS									
	1		2		3		4		Totals	
	No.	%	No.	%	No.	%	No.	%	No.	%
Class 1[1]	66	30.0	27	12.3					93	42.3
2	25	11.4	53	24.1	3	1.3			81	36.8
3			13	5.9	21	9.5			34	15.4
4					7	3.2	5	2.3	12	5.5
	91	41.4	93	42.3	31	14.0	5	2.3	220	100.0
Class 1[2]	263	64.1	15	3.7					278	67.8
2	59	14.4	57	13.9					116	28.3
3			9	2.2	5	1.2			14	3.4
4							2	.5	2	.5
	322	78.5	81	19.8	5	1.2	2	.5	410	100.0

[1] The ratio of methylene blue to milk was 1: 571,000.
[2] The ratio of methylene blue to milk was 1: 100,000.

In the first series of comparisons there were 27 samples that graded Class 2 with the methylene blue test and Class 1 with the resazurin test. From the low resazurin numbers after one hour of incubation it can be reasoned that cells were comparatively few in these milks and bacteria had multiplied very little during the first hour of incubation, but had

* Classification given in Standard Methods of Milk Analysis, p. 41, published by the American Public Health Association, sixth edition, 1934.

multiplied sufficiently thereafter to decolorize the methylene blue within two to five and one-half hours. We feel that the fact that the resazurin test classifies these milks as it does, rather than disqualifying the test is an advantage, since the number of organisms originally present in the milk, that is, its original contamination, is not always correlated with the methylene blue test, especially in good quality milks. Also, the conditions to which the milk is subjected in conducting the methylene blue test differ considerably from those normally used by the consumer, especially in regard to holding temperature.

By making two observations in the resazurin test, the first after one hour of incubation and the second after two hours, a more nearly complete picture of the quality of milk could be obtained. However, this procedure lengthens the time of the test, which is not always desirable. One hour of incubation is generally sufficient to detect most physiologically abnormal and pathological milks, as well as milks containing actively growing organisms. Of course, those milks that contain sluggish organisms that do not start multiplication until after the milk has been incubated for over one hour would escape detection. The test would have to be altered to include more observations at longer incubation periods to enable a proper grading of these types of milk. By applying the resazurin test so that only one observation is taken, considerable time is saved over that required by the methylene blue test.

THE USE OF RESAZURIN TO DETECT SANITARY CONDITION OF HERD MILK SAMPLES

In Table 3 is presented a series of tests on herd milk samples, including microscopic counts, cell counts, methylene blue reduction tests (concentration of 1 to 200,000), and resazurin reduction numbers read every hour for four hours.

While, as previously stated, it is impossible to ascertain the oxidation-reduction potential of a milk-resazurin mixture by its developed color, the rate of change of color should be some indication of its bacterial development. However, due to differences in reducing intensity of organisms and the sensitivity of resazurin to certain unknown factors not necessarily associated with bacterial flora, a true correlation between the rate of resazurin reduction and the rate of bacterial multiplication is not to be expected. Nevertheless, considerable information can be obtained as to the flora of the milk by observing the rate of reduction as manifested in the change of color from blue to pink during incubation.

To illustrate, consider sample No. 734. The resazurin number after one hour of incubation was only 3, indicating very little bacterial multiplication, and the number only increased from 3 to 7 within the next two hours, which still indicated very little bacterial multiplication, but between

TABLE 3

Comparison of resazurin reduction tests with other quality tests on market milk

PATRONS NO.	MICROSCOPIC COUNT IN THOUSANDS	CELL COUNT IN THOUSANDS	METH. BLUE REDUCT. TEST 1 : 200,000 HOURS	RESAZURIN TEST—CHART NUMBERS HOURS INCUBATED—37° C.					
				1	2	3	4	6	7
725	250,000	450	7/12	F					
937	40,000	1,300	1 7/12	20	F				
941	9,000	300	1 3/4	F					
654	7,000	450	2 5/6	15	20	F			
876	6,500	1,600	3	17	F				
839	4,500	1,800	5	7	17	22	F		
924	3,600	380	3 7/12	20	4/5 F	F			
756	3,600	350	6 2/3	13	17	19	4/5 F		F
804	3,500	1,400	3 2/3	15	19	22	F		
821	2,500	730	3 1/3	5	17	4/5 F	F		
771	1,900	540	7 1/6	13	19/11	F/11	4/5 F		
624	1,500	800	3 1/2	11	20	22/F	F		
822	1,500	270	4	5	20	4/5 F	F		
788	760	930	12 1/2	7	9	9	15		F
788	700	1,400	10	6	8	13	15		F 9 hrs.
734	640	540	7 5/12	3	6	7	15		1/2 F
741	600	300	5 1/6	5	15	20	F		
718	590	1,000	8 1/4	2	4	7	11		16
744	590	400	10	3	5	6	9		
915	570	400	11 1/4	3	3	3	4		9
873	560	2,000	7 1/2	12	17	19	19	F	
873	420	820	8 1/2	5	11	15	16		17
842	300	760	9 1/2	5	5	9	13		F 9 hrs.
826	250	1,000	11 1/2	4	11	15	17		18
702	220	1,200	10	3	5	5	7		
814	200	80	11 5/16	2	3	7	7		11
627	190	860	5 1/3	15	19	F/22	F		
914	190	570	10 5/12	3	3	4	11		
687	170	2,400	9 2/3	12	17	19	20		4/5 F
771	160	360	9	5	8	12	13		F 9 hrs.
972	160	580	7 1/4	4	11	19/15	20/17		22
672	140	1,300	5 5/6	3	7	13	20		F
677	140	320	10	2	3	5	11		F 9 hrs.
602	140	800	8	4	7	12		17	
756	130	280	8	2	4	7		12	
750	110	3,200	5 1/12	15	18	20	22		F
934	110	2,500	7 1/2	6	11	15	18		18
755	96	400	8	4	5	7	15		23
877	96	170	7 1/2	3	3	6	7		18
842	80	550	7	3	5	7	16		22
786	64	2,400	8	10	15	17	19		F
810	64	1,200	11 5/6	7	15	17	18		21
662	64	680	8	4	7	12		19	
799	48	1,200	10	5	9	13	15		
701	32	1,400	10 1/4	12	16	18	19		22
876	32	1,200	10	9	15	17	18		
667*	32	620	8 1/6	17	20	21	21		22
677	32	110	11 5/12	3	4	5	11		17
633	16	1,500	4	5	15	16	19		22
618	16	480	8	2	6	12		19	
786	16	300	8	2	5	7		15	
687	16	3,000	10	9	15	17	18		
935	16	2,200	9 3/4	7	15	18	19		21
877	16	1,600	10	5	12	14	15		1/2 F
926	16	1,300	8	4	11	15		15	
754	16	1,300	10	5	8	9	13		
644	16	1,200	9 1/2	5	12	14	17		

TABLE 3—(*Continued*)

Comparison of resazurin reduction tests with other quality tests on market milk

PATRONS NO.	MICROSCOPIC COUNT IN THOUSANDS	CELL COUNT IN THOUSANDS	METH. BLUE REDUCT. TEST 1 : 200,000 HOURS	RESAZURIN TEST—CHART NUMBERS HOURS INCUBATED—37° C.					
				1	2	3	4	6	7
926	16	1,200	11 1/4	6	13	15	18		18
644	16	1,000	9	3	10	15	17		1/4 F
862	16	1,000	8	3	6	11		15	
720	16	930	7 1/2	5	15	17	20		F
702	16	810	9 1/2	3	5	5	12		20
936	16	800	8	2	10	13		21	F
672	16	720	10	2	5	5	5		
764*	16	700	8	7	13	15	17		23
811	16	600	10 2/3	3	5	5	13		15
801	16	520	8	2	4	5		10	
909	16	510	10 1/4	5	5	13	15		17
650	16	380	9	5	13	19/12	19		F
801	16	380		3	4	5	11		15
899	16	280	8	2	6	10		19	
814	16	280	10	2	3	5	6		F 10 hrs.
741	16	240	7 1/2	2	3	5	8		F 6½ hrs.
608*	16	240	8 1/2	9	15	17	18		22
947	16	220	6/11/12	2	3	3	4		11
656	16	200	12 2/3	3	4	7	13		17
792	16	120	8	2	4	6		17	
777	16	112	6 5/6	3	6	15			F

Resazurin numbers greater than 19 indicate partial fading of the pink modification, the larger the number the more faded.

* No. 764—Chloride .137%
608— " .138%
667— " .130%

F—faded

the third and fourth hours the resazurin number changed from 7 to 15. Within this fourth hour the bacterial population must have increased considerably to account for this rapid change in resazurin reduction, but the flora was not of the fast reducing type, since over seven hours elapsed before the dye finally began to fade. The methylene blue sample did not decolorize before 7 5/12 hours. The microscopic count before incubation was 640,000, and in view of this rather high count and low resazurin number after one hour of incubation, many of the organisms must have been in the resting stage or dead.

In several of the samples the resazurin numbers are considerably greater than would be expected from the microscopic count. In most of these samples there are rather high cell counts. In general, wherever there has been a rapid reduction of resazurin within the hour of incubation in samples of milk of low bacterial numbers, either some abnormal feature of physiological or pathological origin has been found or a rapid multiplication has occurred. On the other hand, some abnormal milks have not always produced a marked reduction of resazurin within the first hour of incubation.

RECOMMENDED PROCEDURE FOR CONDUCTING THE RESAZURIN TEST

The following procedure for the resazurin test is suggested. Prepare a .05 per cent aqueous solution of resazurin by dissolving the dye in hot, distilled, sterile water, in a volumetric flask of suitable capacity. Cover the flask and cool, then fill to mark with sterile, distilled water. To keep this stock solution sterile pour approximately the amount desired into a sterile bottle, then flame the neck of the flask, cover with a sterile stopper and place in the dark until the following day or when again needed. Use the solution transferred to the sterile bottle for the day's run. The amount of solution required for each milk sample, which is 0.1 ml. per 10 ml. milk, can be either measured out by using a pipette calibrated in 0.1 ml. divisions, or a pipette with a tip tapered to deliver the required amount in exactly whole drops, *i.e.*, not fractions of a drop.

To eliminate the necessity of tipping the test tubes containing the milk and resazurin solution to effect uniform distribution, the dye solution should be added to the test tube first and the milk then poured in quite rapidly. However, where it is not practicable to follow this technique, the tubes must be turned upside down to obtain uniform mixing. Since the results are only comparative, either method is satisfactory as long as the technique used is consistent.

Incubate the mixtures at 37° C., in a bath kept in a dark portion of the room, or, if this is impossible, cover the bath with a black cloth or any suitable cover which will shut out the light. After one hour of incubation, compare the developed colors with the color chart and from readings obtained group the samples into the following four classes:

Class 1—Good milks, colors between 1 and 6.
2—Fair milks, colors between 7 and 18.
3—Poor milks, colors pink but not faded.
4—Very poor milks, decolorized.

By returning the samples to the bath and recording hourly changes until the dye has decolorized, a more precise analysis of the sanitary conditions of the milks may be obtained. A microscopic examination will aid in arriving at a correct interpretation of the results. See discussion of sample No. 734, as an example.

The following outline enumerates the main conditions that may be encountered:

1. If the reduction is rapid, commencing at or soon after incubation,
   Significance:
   (a) Milks highly contaminated with flora in actively growing state.
   (b) Organisms strongly reducing.
   (c) Pathological milks.
   (d) Physiologically abnormal milks.

2. If the reduction is slow over four to six hours of incubation,
   Significance:
   (a) Low bacterial count.
   (b) High bacterial count with either dead or inactive organisms.
   (c) Low cell count.
   (d) Very little multiplication of organisms.
   (e) Much development of bacteria with weakly reducing characteristics.
3. If the reduction is first slow, then followed by a rapid reduction,
   Significance:
   (a) Physiologically normal milk.
   (b) Not pathological milk.
   (c) Low bacterial count.
   (d) High bacterial count with organisms in lag phase.
   (e) During period of rapid reduction bacteria are on the threshold of or in the logarithmic growth phase and the speed of reduction is a measure of their reducing power.
4. If the reduction is at first rapid and then followed by a slow reduction,
   Significance:
   (a) Physiologically abnormal milks.
   (b) Pathological milks.
   (c) High bacteria count, but poor reducers if well advanced in their logarithmic growth phase.
   (d) Multiplication stopped soon after incubation.
   (e) Very often old milk in which some multiplication had occurred.

Satisfactory colors can be obtained for the color chart by using the dyes described in this paper. However, there are, no doubt, many other dyes, as well as other vehicles, that could be used to produce a chart with colors similar to those developed when resazurin undergoes reduction in milk. Sterilized milk has not been found to be satisfactory as a vehicle, since the yellow color produced during sterilization alters the shades to the extent that they will not match the colors developed during incubation of the mixtures.

The yellow pigment in milk lessens the blue intensity of the resazurin-milk mixtures, the amount depending upon the concentration of pigment. As the yellow pigment, carotene, is fat soluble, it will be carried to the top with the fat, so after the sample has been held for one hour and the fat collected at the top, the milk-resazurin colors approximate more nearly the colors obtained when adding resazurin directly to skim milk. Consequently, the color standards should be made to compare with skim milk-resazurin mixtures rather than with whole milk-resazurin mixtures.

SUMMARY

From the results of this investigation the following conclusions are drawn:

1. Only one hour is required to complete the resazurin test as prescribed in the text, while the methylene blue test requires over five hours.

2. Milk can be classified into four groups as regards sanitary condition by means of the resazurin test.

3. Milks from diseased udders and milks from physiologically abnormal cows have significant effects on the reduction of resazurin, and hence the test aids in their detection.

4. By observing the rate of color change of resazurin-milk mixtures over a period of hours of incubation, considerable information as to the flora can be obtained.

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THE BACTERIOLOGY OF SWISS CHEESE

IV. EFFECT OF TEMPERATURE UPON BACTERIAL ACTIVITY AND DRAINAGE IN THE PRESS

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In the manufacture of Swiss cheese, the heating of the kettle contents to 53° C. or slightly higher, and the high temperature maintained for a number of hours in the press, are known to be essential not only for the proper expulsion of whey from the curd, as pointed out by Orla-Jensen (10), Vas (12), and Koestler (9), but also for the control of bacterial activity. Burri (3) stated that only the thermo-stable organisms survive the heating, with a result that in the press 99 per cent of the surviving lactic acid organisms are either *Streptococcus thermophilus* or *Thermobacterium helveticum*. In a previous paper (6) it was shown that the aerobic gas-forming organisms which are frequently present in milk grew very little if at all in the cheese kettle unless present in large numbers, and that while *Streptococcus lactis* grew early in the kettle it was stopped by the heating process. Of the starter bacteria, *S. thermophilus* (C_3) grew during the early part of the cooking period; the lactobacilli (39a and Ga) not only did not grow but actually decreased in numbers by dipping time.

A study of the growth of the starter bacteria in the press as reported in a previous paper (7) showed that *S. thermophilus* (C_3) resumed active growth within 3 or 4 hours after dipping and continued for 6 to 8 hours before any retardation became evident. *Lactobacillus bulgaricus* (Ga) and *Lactobacillus helveticus* (39a) did not begin to grow until 5 to 10 hours after dipping, the time depending on the culture; the Ga culture, with the higher maximum growing temperature, started to grow earlier in the cheese. This indicated that the starter organisms do not begin to grow until temperature conditions are favorable.

Further observations on bacterial activity in the cheese showed certain significant facts. It was found that at 3 hours after dipping the pH of the interior of the cheese (4 or more inches from the hoop edge) was usually between 5.90 and 6.15, while the pH near the outside of the cheese ($\frac{1}{2}$ to 1 inch beneath the hoop edge) was between 5.60 and 5.85. The more rapid acid production near the hoop edge appeared to be consistent with results reported by Orla-Jensen (11), who showed that whey obtained from the cheese board 3 hours after dipping contained 3 to 4 times as much titratable acid as that obtained from the center of the cheese. His data showed that much of this acid came from used cheese cloths. The higher titratable

acidity at the surface, he believed, was also partially the result of rapid evaporation from the surface of the cheese, although he indicated that other factors may also contribute to this difference. Temperature readings at the center of the cheese were included in these data but temperatures near the perifery were not determined.

In this paper are shown the changes in temperature, bacterial population and pH values which occur at varying distances from the hoop edge toward the center of Swiss cheese during the first 21 hours in the press; data are presented showing the effect of variations in room temperature upon the above factors, the relationship between the production of acid and the expulsion of whey from the cheese, and the effect of these factors upon the quality of the cheese. The bacterial content and acidity of cheese cloths are recorded, and the possible effect of contamination from this source upon the rate of acid production just beneath the perifery of the cheese is discussed.

EXPERIMENTAL PROCEDURE

The experimental work was conducted in part at a factory in Ohio, with cheeses averaging 160 pounds, green weight; more detailed studies were made with laboratory cheeses of about 55 pounds, green weight.

The starters used consisted of pure cultures of *S. thermophilus* (C_3) in combination with pure cultures of either *L. bulgaricus* (Ga or 39aH) or *L. helveticus* (39a), each being grown separately in skim milk or in whey.

The lactobacillus cultures referred to herein have been in use for a number of years in these laboratories and in the factories, and they differ distinctly one from another in morphology, maximum growing temperature and the amount of acid produced in milk; however, the identities of these cultures have not been positively established.

Experience has shown that the medium used for the preparation of starters in factories often is not sterilized adequately, with the result that the starters often are not pure cultures. In these experiments particular care was taken to insure the sterilization of the skim milk or whey used in the preparation of starters and every precaution was taken to prevent subsequent contamination or the use of mixed cultures.

Temperature readings were obtained by inserting standardized thermometers so that the mercury bulbs would be located at the points at which temperatures were to be determined. In making readings at successive 1-inch distances from the hoop edge, a cardboard strip with $\frac{1}{4}$-inch holes 1 inch apart was placed on the top of the cheese along its diameter, the first hole being 1 inch from the circumference. The thermometers were inserted through the holes in the strip and pushed into the cheese until their bulbs were at points equidistant from the top and bottom surfaces.

Samples of cheese for pH, moisture, and bacterial count determinations were obtained by removing a 6-inch plug, by means of a trier, from the hoop

side of the cheese halfway between the top and bottom surfaces. The plug was cut cleanly into 1-inch sections, measuring from the hoop end of the plug. Then $\frac{1}{4}$ inch was cut off from both ends of each section, leaving the middle $\frac{1}{2}$ inch. This was cut into two halves longitudinally. One part was used for the pH determination and the other for the bacterial count. A section from the inner end of the plug, more than 4 inches from the hoop edge, was used for the duplicate moisture determination. The sample used for bacterial counts was ground in a sterile mortar with sodium citrate and water, by the method described by Burkey (1). The direct microscopic count was made from this homogenous sample.

The cooking temperature used in the manufacture of cheese was in all cases 53° C.

RESULTS

The results of temperature determinations, recorded in Table 1, show that the outer area of the cheese cools rapidly and that a decrease of 2° to 4° C. in room temperature results not only in an acceleration of cooling just inside the surface of the cheese during the first 3 or 4 hours but also a correspondingly more rapid cooling in the interior, especially after that time. When the room temperature is low, the temperatures of all parts of the cheese are likewise relatively low at 21 hours; but the difference between that of the interior and that of the outer area is practically the same as when the room temperature is high. In securing the data shown in Table 1, in which are shown average results in 7 factory cheeses made on

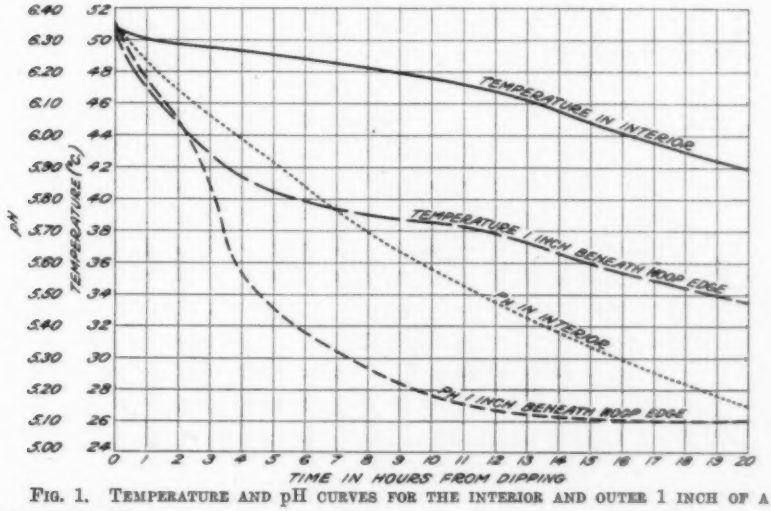

FIG. 1. TEMPERATURE AND pH CURVES FOR THE INTERIOR AND OUTER 1 INCH OF A FACTORY CHEESE.

TABLE 1

Influence of room temperature on the temperature in factory Swiss cheese

NO. OF CHEESES	TEMPERATURE AT DIPPING, °C. (AVE.)				TEMPERATURE AT 1 HR., °C. (AVE.)				TEMPERATURE AT 3 HRS., °C. (AVE.)			
	Room	Cheese			Room	Cheese			Room	Cheese		
		Edge	Top	Center		Edge	Top	Center		Edge	Top	Center
2	29.0	50.5	50.5	50.5	29.3	46.8	47.8	50.1	30.9	42.1	45.6	49.9
2	27.0	49.9	49.9	49.9	27.5	45.3	46.4	49.9	28.8	41.3	44.2	49.4
3	25.0	51.1	51.1	51.1	25.3	45.6	46.7	50.3	27.1	40.9	44.9	48.7

TABLE 1 (Continued)

Influence of room temperature on the temperature in factory Swiss cheese

NO. OF CHEESES	TEMPERATURE AT 5 HRS., °C. (AVE.)				TEMPERATURE AT 7 HRS., °C. (AVE.)				TEMPERATURE AT 21 HRS., °C. (AVE.)			
	Room	Cheese			Room	Cheese			Room	Cheese		
		Edge	Top	Center		Edge	Top	Center		Edge	Top	Center
2	32.3	40.5	44.9	49.4	32.7	40.0	44.2	48.2	24.8	32.8	36.5	46.8
2	30.6	39.0	43.4	48.6	30.4	37.6	42.5	47.2	.....	.....	.....	.....
3	29.3	39.1	44.2	47.3	28.2	37.6	42.6	46.8	21.0	30.0	36.9	42.0

three different days, the room temperatures were recorded at frequent time intervals, and also the temperatures at the following points within the cheeses: at the central point in the interior; 1 inch beneath the rind at the center of the top surface; and 1 inch beneath the rind at the hoop edge, halfway between the top and bottom surfaces.

The typical relationship between the decrease in temperature at different points in Swiss cheese and the development of acidity at these points is shown in Figure 1. These data are from a factory cheese made with $\frac{1}{8}$ per cent of *L. bulgaricus* (Ga) skim milk starter grown at 42.5° C. and $\frac{1}{8}$ per cent of *S. thermophilus* (C_3) whey starter grown at 37°. It will be noted that a rapid decrease in temperature at the hoop edge results in a correspondingly rapid development of acidity.

More detailed results were obtained in a study made of a 55-pound laboratory cheese. The pH, bacterial count and temperature determinations were made at regular intervals of time at successive 1-inch distances from the hoop edge. These data are shown in Table 2. It will be noted that in general the increase in numbers of the starter organisms (C_3 and Ga) and the increased acid production occur as a result of the decrease in temperature at that point in the cheese. C_3 increased rapidly in numbers in the area 1 inch beneath the hoop edge but growth, as shown by increase in numbers, did not occur until several hours later in the interior. Ga increased in numbers in the area 1 inch beneath the hoop edge 1 to 3 hours after

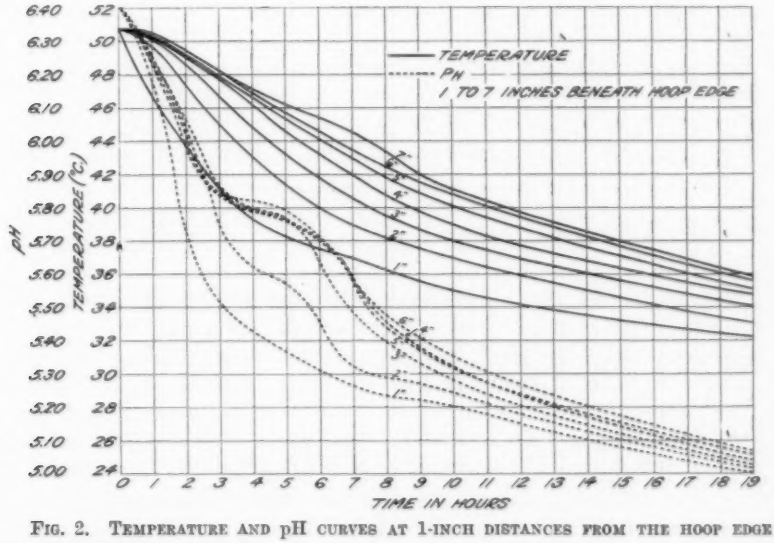

FIG. 2. TEMPERATURE AND pH CURVES AT 1-INCH DISTANCES FROM THE HOOP EDGE OF A LABORATORY CHEESE.

TABLE 2

Temperature, bacterial growth, and acidity at different points in laboratory Swiss cheese (L. bulgaricus and S. thermophilus starters)

TIME	ROOM TEMP.	1 INCH				2 INCHES				3 INCHES			
		Temp.	H-ion Conc.	Organism	Count	Temp.	H-ion Conc.	Organism	Count	Temp.	H-ion Conc.	Organism	Count
hrs.	*°C.*	*°C.*	*pH*		*per gram*	*°C.*	*pH*		*per gram*	*°C.*	*pH*		*per gram*
0	29.7												
1	29.0	46.7	6.18	C_2	250,000,000	49.7	6.23	C_2	104,000,000	50.0	6.22	C_2	91,600,000
				Ga	1,320,000			Ga	198,000			Ga	1,190,000
3	27.1	41.0	5.51	C_2	663,000,000	45.0	5.74	C_2	161,000,000	47.0	5.83	C_2	111,000,000
				Ga	25,500,000			Ga	5,000,000			Ga	7,500,000
5	29.8	38.2	5.37	C_2	873,000,000	41.3	5.57	C_2	154,000,000	43.2	5.77	C_2	124,000,000
				Ga	278,000,000			Ga	73,800,000			Ga	20,500,000
7	30.8	36.9	5.26	C_2	1,580,000,000	39.0	5.32	C_2	425,000,000	40.6	5.48	C_2	174,000,000
				Ga	371,000,000			Ga	462,000,000			Ga	258,000,000
9	29.8	35.5	5.22	C_2	923,000,000	37.5	5.27	C_2	398,000,000	38.7	5.33	C_2	322,000,000
				Ga	269,000,000			Ga	437,000,000			Ga	599,000,000
19		32.2	5.00			33.0				34.0			

TABLE 2—(*Continued*)

TIME	ROOM TEMP.	4 INCHES				5 INCHES				6 INCHES			
		Temp.	H-ion Conc.	Organism	Count	Temp.	H-ion Conc.	Organism	Count	Temp.	H-ion Conc.	Organism	Count
hrs.	*°C.*	*°C.*	*pH*		*per gram*	*°C.*	*pH*		*per gram*	*°C.*	*pH*		*per gram*
0	29.7	50.7	6.41	C_2	116,000,000								
				Ga	1,300,000								
1	29.0	50.1	6.24	C_2	143,000,000	50.3	6.25	C_2	60,300,000	50.4	6.24	C_2	99,500,000
				Ga	1,360,000			Ga	207,000			Ga	3,590,000
3	27.1	47.8	5.84	C_2	105,000,000	47.9	5.84	C_2	119,000,000	48.2	5.86	C_2	92,700,000
				Ga	198,000			Ga				Ga	2,330,000
5	29.8	44.5	5.79	C_2	83,300,000	45.4	5.76	C_2	63,600,000	45.8	5.76	C_2	76,500,000
				Ga	1,180,000			Ga	1,980,000			Ga	3,030,000
7	30.8	42.1	5.59	C_2	142,000,000	43.0	5.60	C_2	139,000,000	43.5	5.58	C_2	140,000,000
				Ga	161,000,000			Ga	192,000,000			Ga	89,500,000
9	29.8	39.8	5.38	C_2	246,000,000	40.8	5.37	C_2	156,000,000	41.5	5.41	C_2	168,000,000
				Ga	787,000,000			Ga	502,000,000			Ga	380,000,000
19		34.7				35.0	5.07			35.6			

dipping; in the 2-inch area in 5 hours, and at 3, 4, 5, and 6 inches no increase in numbers was evident until the sixth or seventh hour after dipping. The temperature and pH curves for the same cheese, presented in Figure 2, demonstrate the rapid decrease in temperature in the outer 1-inch area, the progressively less rapid decrease at increasing distances from the hoop edge, and the corresponding increase in acid with the decrease in temperature. It may be seen from these results that a sample to be representative of the interior of the cheese should be obtained at a point at least 4 inches from the hoop edge. Each hump in the pH curves in Figure 2 represents a diminution of acidity which may occur when the growth of C_3 is retarded before the lactobacilli begin to grow.

Data from a similar study of laboratory cheeses made with a different lactobacillus culture are shown in Table 3. The starters used were *L. helveticus* (Bergey, 1934) (39a) and *S. thermophilus* (C_3). These results further demonstrate the early growth of C_3 throughout the cheese. 39a began to grow about 3 hours after dipping in the area 1 inch from the hoop edge, but there was no increase in numbers in the interior of the cheese until the ninth hour.

TABLE 3

Bacterial growth and acidity in laboratory Swiss cheese (L. helveticus and S. thermophilus starters)

NO.	HOURS AFTER DIPPING	1 INCH BENEATH HOOP EDGE			INTERIOR OF CHEESE		
		pH	Counts per gram		pH	Counts per gram	
			39a	C_3		39a	C_3
1	1	6.29			6.36	5,440,000	39,700,000
	3	5.56	5,430,000	888,000,000	6.07	6,940,000	38,900,000
	5	5.28	48,700,000	849,000,000	5.82	2,950,000	207,000,000
	7	5.21	193,000,000	1,410,000,000	5.44	1,200,000	362,000,000
	9	5.15	355,000,000	1,380,000,000	5.39	3,050,000	453,000,000
	21	4.98			5.00	367,000,000	667,000,000
2	7	5.23	245,000,000	790,000,000	5.48	2,650,000	273,000,000
	11	5.01	533,000,000	1,200,000,000	5.36	63,200,000	158,000,000

The effect of combinations of starters of different activities on bacterial growth and pH in the outer 1-inch area and in the interior of factory cheese is shown in Table 4. The starters used in the 3 cheeses were: No. 1, $\frac{1}{12}$ per cent of *L. bulgaricus* (39aH) skim milk grown at 46° C. for 12 hours and $\frac{1}{8}$ per cent of C_3 whey grown at 36.5° for 12 hours; No. 2, $\frac{1}{12}$ per cent of 39aH whey grown at 43° for 12 hours and $\frac{1}{8}$ per cent of C_3 whey grown at 38.5° for 12 hours; No. 3, $\frac{1}{12}$ per cent of 39aH whey grown at 43° for 12 hours and $\frac{1}{8}$ per cent of C_3 skim milk grown at 36.5° for 12 hours.

The data show that C_3 and 39aH are more active in the cheese when grown in skim milk than when grown in whey.

TABLE 4

The effect of L. bulgaricus (39aH) and S. thermophilus (C_2) starters of different activities on bacterial growth, acidity and final results in factory Swiss cheese

CHEESE NO.	KIND OF STARTERS	HOURS AFTER DIPPING	1 INCH BENEATH HOOP EDGE			INTERIOR OF CHEESE			DRAINING APPEARANCE 21 HOURS	FINAL SCORE
			Counts per gram		pH	Counts per gram		pH		
			39aH	C_2		39aH	C_2			
		0	.....	.....	6.34	422,500	9,362,000	6.34		
	39aH in	1¼	35,000	21,056,000	5.91	227,050	12,301,000	6.15	Fairly dry;	
1	skim milk	3½	28,160,000	429,000,000	5.50	1,500,000	124,000,000	5.90	slightly	82
	C_2 in whey	10	1,433,600,000	538,200,000	5.04	444,500,000	161,290,000	5.65	rubbery	
		22	.....	.....	.....	.....	.....	5.20		
		0	.....	.....	6.39	173,250	17,136,000	6.39		
	39aH in	1¼	35,000	21,350,000	6.04	136,000	20,336,000	6.23		
2	whey	3½	6,200,000	566,000,000	5.58	1,500,000	96,000,000	6.05	Slightly wet;	
	C_2 in whey	10	481,650,000	760,300,000	5.32	369,180,000	245,700,000	5.55	leaky, short	78
		22	.....	.....	.....	.....	.....	5.33		
		0	.....	.....	6.33	176,000	23,296,000	6.33		
	39aH in whey	1¼	34,100	34,100,000	5.82	102,300	40,000,000	5.87	Driest;	
3	C_2 in skim	3½	20,480,000	395,520,000	5.50	1,500,000	238,000,000	5.60	rubbery,	95½
	milk	10	390,610,000	139,190,000	5.08	172,720,000	301,625,000	5.52	more firm	
		22	.....	.....	.....	.....	.....	5.10		

In this experiment the rapid acid production in the interior of cheese No. 3, especially during the first 3 hours, resulted in more extensive drainage and in a higher scoring cheese than was obtained in cheeses Nos. 1 and 2. This would indicate that the basis for proper drainage is established during the first few hours after dipping. It is evident that *S. thermophilus* (C_3), which is the active starter during this period, performs a significant rôle in the drainage of Swiss cheese (2). Furthermore, these results indicate that the proper combination of starters to use depends upon the activity of each starter. The more active the lactobacillus starter the greater is the need for an active *S. thermophilus* starter to facilitate drainage from the interior of the cheese.

A great difference in the rate of cooling between the interior and the outer 1-inch area causes a correspondingly great difference in the rate of acid production and thus causes expulsion of a small proportion of the whey. In 60 laboratory cheeses there were 16 cheeses in which the difference in pH at 3 hours between the interior and the area 1 inch beneath the hoop edge was less than 0.30, with an average percentage of moisture in 21 hours of 38.02; 21 cheeses with a difference in pH of 0.30 to 0.40, with an average percentage of moisture of 38.39; and 23 cheeses with a difference in pH of over 0.40, with an average percentage of moisture of 38.58. The drier cheeses had the higher average scores.

In these 60 cheeses, determinations of moisture both in the interior and in the area 1 inch beneath the hoop edge were made at 21 hours. In 48 of the 60 cheeses, the percentage of moisture was greater just under the rind than in the interior. This tendency for moisture to collect just beneath the surface is a possible explanation for the occurrence of the defective whitish, grainy, and checked area that is often found just beneath the rind.

ACIDITY AND BACTERIAL COUNTS OF CHEESE CLOTHS

In connection with studies of acidity it was found that at dipping the pH of the whey on the top of the cheese and in contact with the dipping cloth was 6.32, while that of the whey obtained from within the cheese was 6.45. Orla-Jensen (11), in work referred to earlier in this paper, found that acidity in used cheese cloths materially increased the titration values of the whey as it flowed from the cheese boards. It was therefore considered desirable to study the effect of the cloths upon bacterial activity in the cheese.

In order to study the amounts of acid contained in dipping cloths, 2 cloths were each soaked for 10 minutes in 1,500 cc. of sterile distilled water. The rinse-waters thus obtained, when titrated with phenolphthalein as an indicator, were found to contain 0.078 and 0.065 per cent acid, respectively.

In order to determine whether the bacteria present in the cloths used on the cheese had any influence on the rate of acid development at the sur-

face of the cheese, two cloths were used on cheeses and then washed as usual; one was dried in the usual manner after washing, and the other was thoroughly autoclaved before drying. On the following day these cloths were each soaked in 500 cc. of sterile distilled water for 10 minutes. All but a small amount of this water was absorbed by the cloths. In the remaining water the counts of organisms per cc. were as follows: (1) from unsterilized cloth: 1,000 yeasts, 10,000,000 streptococci, and 100,000 rods; (2) from autoclaved cloth: 10 yeasts, 1,000 streptococci, and 10 rods. The unsterilized or regular cheese cloth contained large numbers of bacteria, particularly of the *S. thermophilus* type, as compared with the low counts in the autoclaved cloth. These two cloths were used on two factory cheeses at the second turning. No difference was found in the rates of acid development at the surfaces of the two cheeses.

DISCUSSION

The temperature of Swiss cheese in the press has been found to decrease rapidly in the outer 1-inch area during the first 3 or 4 hours after dipping. The rate of cooling becomes less as the distance from the hoop edge toward the center becomes greater. After the fifth hour all parts of the cheese cool at a fairly uniform rate, the temperature reaching 36° to 46° C. in the interior of the cheese in 21 hours. The decrease in temperature is comparatively large in small cheese and in those held at a low room temperature.

The rapid cooling in the outer 1-inch area, as compared with that in the interior, results in a corresponding increase in bacterial growth and acid production near the surface. When the difference in pH at 3 hours between the outer area and the interior is large, *e.g.*, more than 0.30, the cheese tends to retain moisture and is therefore likely to show checking near the rind or a splitting of the curd known as "glass."

Experimental data presented herein show that acid production, as shown also in paper No. II of this series (7), and the extent of drainage in the interior of the cheese during the first 4 to 6 hours, are due largely to the growth of *S. thermophilus*. The lactobacilli do not start to grow in the interior of the cheese until 5 to 10 hours after dipping, when the temperature conditions become favorable. *L. bulgaricus* (39aH and Ga) having a higher maximum growing temperature begins to grow in the interior of the cheese at about the fifth or sixth hour, but *L. helveticus* (39a) does not begin to grow until the ninth hour or later.

In the outer 1-inch area, however, *S. thermophilus* begins to grow soon after the cheese is placed in the press; Ga and 39aH about an hour later; and 39a about 3 hours after dipping. Consequently, there is a tendency for acid production to start quickly and proceed rapidly near the perifery. The increase in acidity hastens the expulsion of whey from the cheese particles, causing them to cohere more closely and finally to mat together. As a result, the more rapidly acid is produced near the perifery, the sooner there

is formed a matting of the cheese particles, causing in a few hours the gradual formation of a layer about the cheese which, as time goes on, becomes an almost impervious barrier through which whey can pass only very slowly. If, therefore, the lactobacilli are very active and *S. thermophilus* weak or low in numbers, an excessive amount of whey is retained and it tends to collect near the rind. This causes the cheese to be wet and to "leak" in the press, resulting finally in the defects referred to above. When, therefore, an active Ga or 39aH starter is used, a particularly active *S. thermophilus* starter should be employed to hasten drainage from the interior of the cheese. The use of a lactobacillus starter such as 39a, which produces delayed activity in the cheese, requires the use of a *S. thermophilus* starter which will produce prolonged activity so that the production of acid will be continued until the lactobacilli begin to grow.

It has been pointed out (5) that *S. thermophilus* probably always occurred naturally in the starter or has had its source either in the milk or cheese equipment or in the milk itself. Reports of investigators in Switzerland show the presence of a streptococcus in the natural rennet from stomachs. Our observations, based on several direct isolations, have shown that *S. thermophilus* is often present as a contamination of the lactobacillus cultures as handled in the cheese factories. Due to its relatively high thermal death point, *S. thermophilus* withstands the usual washing and sterilizing of cheese equipment. The equipment is invariably heavily seeded with this organism, which may thus gain entrance into the cheese day after day. An examination of the fresh milk also shows the presence of this organism, especially if whey is returned to the farmers in milk cans. The contamination by *S. thermophilus* from these sources may be so great that it may be necessary to add only a small amount of the streptococcus starter.

The rate at which drainage occurs must also be given careful consideration. Slow biological activity in the interior results in slow drainage of whey from the cheese and provides conditions favorable for the growth of gas-forming bacteria, which if present in large numbers may cause the pressler defect, or an excessive amount of acid may be formed later in the press and as a result, in the early stages of ripening, normal eye-formation may be retarded and the cheese may be "blind" or the defects known as "glass" and rind-checking may occur. If acid production and drainage progress too rapidly during the early hours on the press (pH 5.75 or less at 3 hours), the further production of acid is likely to be deficient (pH 5.30 or more at 21 hours), and the cheese body is likely to be short and crumbly and the curd poorly knit, as pointed out in papers II (7) and III (8) of this series. Such a cheese tends to take on "set" too early and to become "overset," and the eyes are likely to be "cabbagy" and irregular in shape. Koestler (9) has pointed out the harmful effects of too little and too much acidity in the press, from the standpoint of effects upon curd properties and upon the ripening and the final quality. Dorner (4) has concluded

that it is desirable to so regulate the use of starters as to provide a rate of acid production which is neither too rapid nor too slow, thereby promoting adequate drainage and improved quality of cheese.

The drainage of Swiss cheese results in the loss of other substances in addition to moisture. These substances undoubtedly include such readily available food for bacterial growth as lactose, soluble proteins and certain colloidal nitrogenous material. The amounts of these substances retained in the cheese undoubtedly depend in part on the rate and amount of drainage. If drainage progresses too rapidly there is apt to be a deficiency of these food materials for the proper growth of the starter and eye-forming bacteria. At the same time other bacteria, due to their less fastidious natures, can grow and may cause abnormal eye-formation and the other defects referred to above. On the other hand, if drainage is retarded, larger amounts of these food materials are apt to be retained, causing too great bacterial activity. In this case the excessive amount of acid formed is likely to retard eye-formation and influence the normal ripening process unfavorably. Additional work is needed to show what effect drainage has upon eye-formation and upon the final ripening of Swiss cheese.

The proper use of starters will provide sufficient numbers of *S. thermophilus* of the proper activity to reduce the pH in the interior to about 6.00 in 3 hours, as shown in papers II (7) and III (8) of this series, or a pH decrease of 0.30 to 0.40 in that time, and will provide sufficient numbers of active lactobacilli to continue the decrease in pH at a fairly uniform rate so that the pH may reach 5.00 to 5.15 at 21 hours. This 21-hour pH reading may tend to be misleading the nearer it approaches 5.00 or slightly below, since this is about the maximum acidity reached in Swiss cheese. In order to be certain of a uniform decline in pH, it is believed desirable either to make a pH reading in 16 to 18 hours after dipping or to work toward a pH value of 5.10 to 5.15 at 21 hours.

SUMMARY

Temperature, pH, and bacterial counts were determined in Swiss cheese in the press at regular time intervals from dipping to 21 hours in samples taken at definite distances from the outer edge of the cheese. Results were obtained from studies of 55-pound laboratory cheeses and also from large factory cheeses. Moisture determinations were made on samples from the laboratory cheeses. The following conclusions were drawn from the results:

1. The area just beneath the rind cools more rapidly than the interior.

2. Bacterial growth and acid production correspond in general with the decrease in temperature of each part of the cheese.

3. While all the starter bacteria begin to increase in numbers in the area 1 inch beneath the rind during the first few hours after dipping, the initiation of growth in the interior was shown to occur at the following intervals of time from dipping: *S. thermophilus* (C_3), within 2 or 3 hours;

L. bulgaricus (Ga and 39aH), after 5 or 6 hours; and *L. helveticus* (39a), after 9 or 10 hours.

4. Essential functions of *S. thermophilus* in Swiss cheese in the press are the production of acid and the consequent facilitation of drainage from the interior of the cheese during the first 4 to 6 hours after dipping.

5. Large differences in pH between the interior and the area just beneath the rind result in insufficient drainage and high moisture content, and may tend to cause such defects as checking near the rind or a splitting of the curd known as "glass."

6. Bacterial content of cheese cloths was found not to influence the rate of acid production near the surface of the cheese.

7. Samples from the interior of the cheese, in order to yield results representative of the interior of the cheese, should be obtained at points at least 3 or 4 inches from the hoop edge.

8. A judicious use of starters promotes sufficient acid production throughout the cheese and thereby aids in uniform drainage.

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COMBINATION OF CATALYSTS TO REDUCE DIGESTION TIME IN THE DETERMINATION OF NITROGEN

II. Dairy Products

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INTRODUCTION AND HISTORICAL REVIEW

The time required for the determination of nitrogen has been materially reduced since Kjeldahl (1) first proposed his original method. This reduction has been made possible by introducing into the reaction mixture potassium sulphate and catalysts such as mercury and copper. Just recently, the use of selenium has been advocated as a catalyst. Although Lauro (2) was the first to investigate the use of selenium, this substance has been combined with other catalysts by several investigators (3 to 11 inc.) Of these workers, several (7, 8, 10) have found this element to reduce materially the amount of nitrogen obtained in the analysis. Sandstedt (11) also has found with selenium some loss of nitrogen on long heating.

Nearly all of the investigations of the determination of nitrogen in which selenium has been used have been with cereal products. In this communication, there are reported the results obtained when selenium is used with other catalysts in the determination of nitrogen in dairy products. No experimental data are available on the efficiency of selenium when combined with copper, mercury, and strong hydrogen peroxide.

METHODS AND EXPERIMENTAL DATA

The method employed was the Gunning modification of the original Kjeldahl method, which is official with the Association of Official Agricultural Chemists (12). Given amounts of each dairy product were weighed or measured into ten digestion flasks. In case of cheese and milk powder, one gram was used. With the liquid products, the weight was determined by multiplying the cubic centimeters by the specific gravity. Five cc. of evaporated milk and ten cc. of fresh milk were used. After the dairy product had been added to the digestion flask, ten grams of nitrogen-free potassium sulphate and twenty cc. of concentrated sulphuric acid were added. The following catalysts or combination of catalysts were separately added to each of the nine digestion flasks, the tenth being used as a control without catalysts.

(1) One cc. of thirty per cent hydrogen peroxide was added at the beginning of the heating process and then each five minutes during the digestion until the solution cleared.

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TABLE 1
Determination of proteins in dried milk

CATALYST	SKIMMED MILK POWDER		PROTEIN MILK POWDER		LACTIC ACID SKIMMED MILK		WHOLE LACTIC ACID MILK POWDER		WHOLE MILK POWDER		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H_2O_2	47	36.14	61	35.73	53	34.99	39	24.62	84	23.16	56.8	30.93
HgO	25	35.73	30	35.34	26	35.65	20	24.81	22	23.93	24.6	31.09
$HgO + H_2O_2$	24	35.70	32	35.15	21	35.15	16	24.57	24	24.14	23.4	30.94
Se.	27	35.73	31	35.22	23	35.28	26	24.86	28	23.78	27.0	30.97
$Se. + H_2O_2$	28	35.86	27	35.41	20	35.48	20	24.98	20	24.20	23.0	31.19
$CuSO_4$	38	35.80	24	34.77	27	35.18	22	24.90	22	23.99	26.6	30.93
$CuSO_4 + H_2O_2$	29	35.78	25	34.97	29	34.97	17	24.52	23	23.78	24.6	30.80
$HgO + Se. + CuSo_4$	13	35.86	12	35.34	10	35.22	11	24.55	13	24.08	11.8	31.01
$HgO + Se. + CuSO_4 + H_2O_2$	11	35.93	10	35.41	9	34.97	12	25.06	8	23.80	10.0	31.03
Control	97	35.43	92	34.83	129	35.28	75	24.81	101	24.20	98.8	30.91
Average		35.80		35.22		35.22		24.77		23.91		30.98

TABLE 2

Determination of proteins in fluid milk

CATALYST	WHOLE MILK NO. 1		WHOLE MILK NO. 2		WHOLE MILK NO. 3		WHOLE MILK NO. 4		BUTTER MILK		SKIMMED MILK		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	protein Per cent	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H_2O_2	43	3.39	65	3.73	45	3.39	47	3.45	30	3.09	42	3.59	45.3	3.44
HgO	26	3.48	28	3.74	21	3.48	27	3.54	16	3.03	18	3.68	22.7	3.49
$HgO + H_2O_2$	20	3.49	26	3.76	18	3.47	24	3.50	13	3.04	15	3.67	19.3	3.49
Se.	24	3.38	28	3.71	21	3.36	24	3.47	21	3.01	26	3.69	24.0	3.44
$Se. + H_2O_2$	20	3.45	21	3.69	21	3.42	22	3.49	20	3.05	20	3.66	20.7	3.46
$CuSO_4$	23	3.38	27	3.64	24	3.40	20	3.45	17	2.98	22	3.62	22.2	3.41
$CuSO_4 + H_2O_2$	20	3.36	19	3.75	20	3.43	21	3.42	18	2.96	19	3.64	19.5	3.43
$HgO + Se. + CuSO_4$	12	3.48	14	3.71	14	3.48	17	3.48	11	3.03	14	3.77	13.7	3.49
$HgO + Se. + CuSO_4 + H_2O_2$	11	3.49	12	3.75	13	3.46	15	3.47	8	3.01	10	3.72	11.5	3.48
Control	119	3.44	118	3.74	119	3.42	169	3.50	90	2.97	151	3.71	127.7	3.46
Average		3.43		3.72		3.43		3.48		3.02		3.68		3.46

TABLE 3

Determination of proteins in evaporated and condensed milk

CATALYST	EVAPORATE MILK NO. 1		EVAPORATE MILK NO. 2		EVAPORATE MILK NO. 3		EVAPORATE MILK NO. 4		EVAPORATE MILK NO. 5		CONDENSED MILK NO. 1		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H_2O_2	45	6.66	42	6.57	48	7.02	43	7.02	55	7.21	70	7.85	50.6	7.06
HgO	26	6.81	27	6.48	28	7.08	28	7.08	23	7.08	20	8.10	25.3	7.11
$HgO + H_2O_2$	22	6.79	23	6.63	23	7.22	28	7.21	19	7.21	21	7.97	22.7	7.17
Se.	24	6.82	28	6.57	31	7.08	26	7.02	26	7.14	29	7.91	27.3	7.09
$Se. + H_2O_2$	22	6.73	27	6.63	29	7.04	24	7.08	26	7.21	26	7.94	25.7	7.11
$CuSO_4$	19	6.55	30	6.57	27	7.02	23	6.96	26	7.20	27	7.68	25.3	7.00
$CuSO_4 + H_2O_2$	20	6.74	24	6.59	27	7.08	26	7.02	21	7.08	26	7.72	24.0	7.04
$HgO + Se. + CuSO_4$	14	6.71	16	6.63	16	7.22	14	7.08	10	7.12	16	7.91	14.3	7.11
$HgO + Se. + CuSO_4 + H_2O_2$	11	6.63	10	6.63	13	7.27	11	7.02	9	7.14	13	7.78	11.2	7.08
Control	89	6.72	92	6.57	90	7.22	101	7.14	95	7.14	61	7.85	88.0	7.11
Average		6.72		6.59		7.13		7.06		7.15		7.87		7.09

TABLE 4

Determination of proteins in cheese

CATALYST	LEGHORN		BRICK		HOLLAND		SWISS		AMERICAN CREAM		ROQUEFORT		AVERAGE	
	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein	Minutes for digestion	Per cent protein
H_2O_2	46	28.80	50	27.60	54	30.10	60	27.63	56	28.77	47	14.36	52.2	26.21
HgO	28	28.77	18	27.31	29	29.98	25	27.29	33	28.45	29	14.55	27.0	26.06
$HgO + H_2O_2$	30	29.47	19	27.33	26	30.32	20	27.33	30	28.71	24	14.39	24.8	26.26
Se.	24	28.90	23	27.60	29	29.86	23	27.00	38	28.57	29	14.23	27.7	26.03
$Se. + H_2O_2$	24	29.03	23	27.33	27	29.92	21	27.29	34	28.77	25	14.23	25.7	26.10
$CuSO_4$	28	28.96	29	26.52	27	30.30	24	27.85	26	28.20	26	14.04	26.7	25.98
$CuSO_4 + H_2O_2$	24	28.64	27	27.31	25	29.98	19	27.33	25	28.71	25	14.20	24.2	26.03
$HgO + Se. + CuSO_4$	15	28.95	14	27.60	12	30.31	9	27.60	10	28.74	13	14.06	12.2	26.21
$HgO + Se. + CuSO_4 + H_2O_2$	16	29.09	10	27.31	9	30.05	7	27.85	8	28.71	10	14.02	10.0	26.17
Control	87	29.47	103	26.55	135	30.17	94	27.63	123	27.98	86	14.29	104.7	26.02
Average		29.01		27.25		30.10		27.48		28.56		14.24		26.11

(2) Five-tenths gram of HgO.

(3) Five-tenths gram of HgO with hydrogen peroxide added as in (1).

(4) Two-tenths gram of selenium.

(5) Two-tenths gram of selenium with hydrogen peroxide added as in (1).

(6) One gram $CuSO_4 \cdot 5\ H_2O$.

(7) One gram $CuSO_4 \cdot 5\ H_2O$ with hydrogen peroxide added as in (1).

(8) Three-tenths gram HgO, 0.1 gram selenium, and 0.5 gm. $CuSO_4 \cdot 5\ H_2O$.

(9) The same catalysts as in (8) with hydrogen peroxide added as in (1).

The contents of each flask were digested over electrically-heated plates, all units of which were of the same construction and gave the same amount of heat. Each sample was heated until the liquid cleared.

The records of the digestion times and the amount of proteins obtained are given for each dairy product in Tables 1, 2, 3, and 4.

A study of the Tables shows that when strong hydrogen peroxide was used with $CuSO_4$, HgO, and selenium separately, there was only a slight reduction in the digestion time over that obtained without the hydrogen peroxide. The accuracy of the protein determination was not affected. The combination of copper, mercury, and selenium materially reduced the time of digestion over that obtained when each catalyst was used separately. The addition of strong hydrogen peroxide to the mixture of these three catalysts still further reduced the time of digestion, but not enough to warrant the additional trouble and expense.

In every case where $CuSO_4$ was used, either alone or with hydrogen peroxide, the results for protein seem to be slightly lower than those obtained with other catalysts.

CONCLUSIONS

1. Hydrogen peroxide when used with copper, mercury, and selenium (separately or in combination) reduces somewhat the digestion time and does not impair the accuracy of the determination.

2. The combination of the three catalysts (Cu, Hg, and Se) showed a reduction in digestion time from 105 to 13 minutes (averages) when compared to that of the Gunning method. When hydrogen peroxide was used with these three catalysts, the reduction in time was from 105 to 10.7 minutes (averages).

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THE RELATION OF MASTITIS TO RENNET COAGULABILITY AND CURD STRENGTH OF MILK*

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INTRODUCTION

Various estimates have been made as to the prevalence of mastitis among cows that furnish milk to our regular commercial outlets. As early as 1888 Hess, Schaffer and Bondzynski (1) classed udder inflammations with diseases that most commonly afflict domestic animals. Minett (2) in 1930, Seelemann (3) in 1932, and Rosell (4) in 1933 reviewed publications showing the prevalence of mastitis in various sections of the world; citations showing from 15 to 40 per cent of the cows infected with mastitis are common, and in some cases the majority of cows were reported as afflicted. Seelemann points out that the widespread prevalence of mastitis is usually unsuspected. Dairymen are likely to feel that in their respective communities the disease is much less common, but when surveys are made they usually show its prevalence to be in harmony with published results.

While the exact estimates may differ, it is clearly evident that an appreciable portion of our regular commercial milk comes from cows afflicted with subclinical mastitis. Unquestionably many investigations of dairy problems in the past have been complicated by the inclusion of such unsuspected abnormal milk. Fortunately such investigations still are typical of commercial conditions, but we should also understand dairy problems in relation to strictly normal milk so that we may anticipate the benefits or even new problems that may arise from the elimination of milk from mastitis udders. The present study was undertaken to determine the relation of mastitis to the rennet coagulability and curd strength of milk.

REVIEW OF LITERATURE

In 1932 Monier and Sommer (5) in a paper before the American Dairy Science Association reported that two samples of milk which had been selected and studied as typical soft-curd milk were later found from the veterinarian's record to have shown a history of mastitis during the time the samples were being studied. Further work then undertaken showed that while mastitis was not the sole cause of soft-curd milk, it did lower the curd strength. In 1933 Doan and Welch (6) confirmed this conclusion and attributed the lower curd strength of milk from infected udders mainly to the lower casein content of such milk. In 1934 Hansen, Theophilus,

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Atkeson and Gildow (7) reported results to show that mastitis caused by streptococcic infection invariably lowered the curd strength, but that staphlococcic infections did not affect the curd strength appreciably.

As early as 1888 Hess *et al.* (1) emphasized the damaging effect of milk from infected udders on cheese making. Koestler (8) in 1921 noted the slower coagulation of such milk with rennin, and in 1926 Koestler *et al.* (9) reported a higher protein and fat loss in the whey and a more milky appearing whey in making cheese from such abnormal milk. Hill in 1932 (10) compared soft-curd and hard-curd milk in cheese making; he found a higher fat loss in the whey with the soft-curd milk but there was no significant difference in the casein content of the whey. The protein content of the soft-curd milk was 3.33 per cent as compared with 3.8 per cent in the hard-curd milk.

PROCEDURE

The milk samples used in this study were obtained from the cows in the University of Wisconsin Herd which had for some time been segregated into "streptococcus-free" and "infected" groups on the basis of close veterinary supervision which involved udder inspection by manipulation, and on the basis of the following tests applied to the milk: strip cup test, chlorine content by direction titration, brom cresol purple test, catalase test, rennet test, plate colony count on blood agar. Where streptococci were found, tests were made to identify them further, and in all cases the organisms were found to belong to the alpha-group. These tests were in progress throughout this study, and diagnoses were being made entirely independent of it, with the aim of securing a herd that was strictly normal; the "infected" group, as a result, included some cows for which the diagnosis was doubtful. All the cases of mastitis included were comparatively mild; the milk samples in all cases, even where quarter samples were studied, were normal in appearance to casual or untrained observers and in ordinary herd practice would in all probability have been included in the regular milk supply.

The curd strength tests were made according to the Hill (11) procedure, modified as to the method of mixing the milk and coagulant, and as to the equipment used. Mixing was accomplished by discharging the previously tempered milk sample from a 100 cc. pipette with an unusually large delivery opening (water at 20° C., discharge time 4.4 sec.), into the jar that already contained the coagulant, giving the jar a rotary motion in the meanwhile and directing the stream towards the lower corner of the jar. The same curd knife was used for all tests, reversing the blades on the stem for a downward thrust. Using equipment as illustrated in Figure 1, the jar and its contents were placed on the scale platform and the movable dial adjusted to bring the "zero" reading under the indicator; with the curd knife in position directly over the coagulated milk, the air escape was then opened

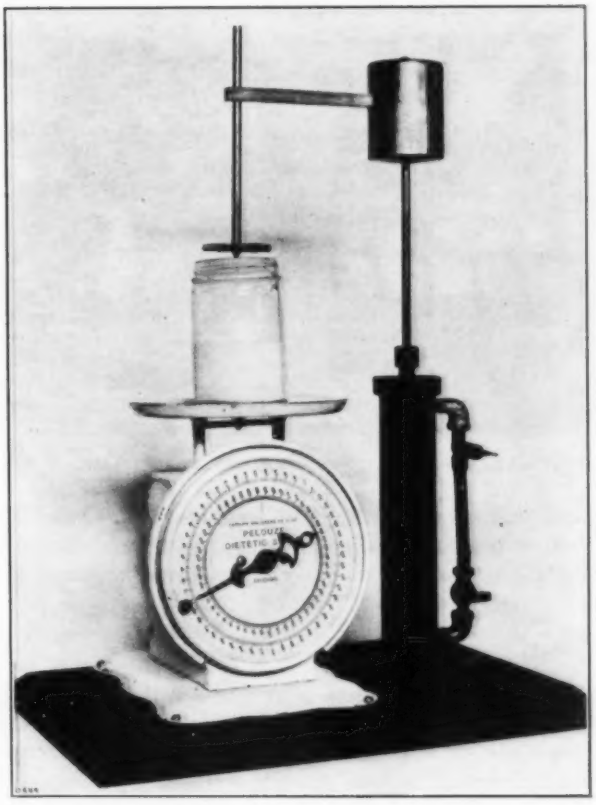

FIG. 1. EQUIPMENT USED FOR THE MODIFIED HILL CURD STRENGTH TEST.

to permit the curd knife to descend at a constant rate as regulated by a needle valve control on the air escape. The resistance encountered by the knife in passing through the curd could then be read off the dial directly.

The coagulation time with rennin was determined on 50 cc. of milk at 30° C. with 1 cc. of a 1 to 50 dilution of commercial rennet extract added. Wide mouth bottles with a capacity of 135 cc. were used (inside dimensions; body-diameter 4.8 cm., height 8 cm., neck-diameter 3 cm.). Equipment as illustrated in Figure 2 was used for tempering the samples, maintaining the temperature during the test and timing the coagulation. During the test the bottles rested on rollers at an angle of approximately 20 degrees from the horizontal and were immersed in the water up to the mouth. The rollers revolved to impart motion to the bottles at the rate of about 8 R.P.M. When the film of milk that clings to the inside surface of the inclined,

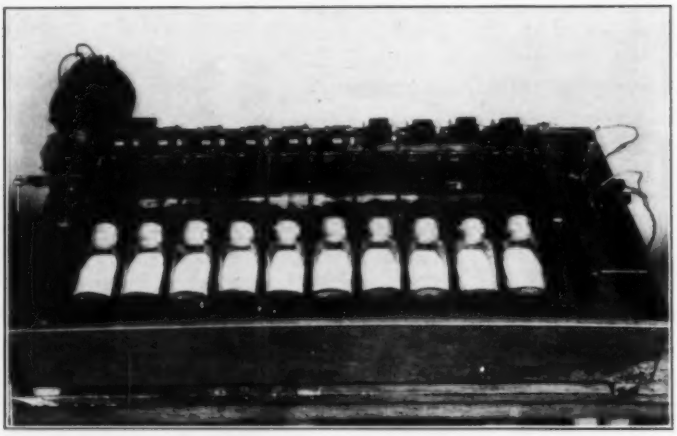

FIG. 2. EQUIPMENT USED FOR DETERMINING THE RENNET COAGULATION TIME OF MILK.

revolving bottle showed the first signs of graininess the test was terminated. With ten samples being timed simultaneously, the timing was accomplished conveniently by engaging and disengaging revolution counters at the start and end of the respective tests.

EXPERIMENTAL RESULTS

A number of curd strength tests were made on the milk from the individual cows in the normal and abnormal herds. Later the coagulation time with rennet was also determined. Table 1 gives a summary of the results. Samples on which both tests were run are averaged separately in Columns 5, 6 and 7, but are also included in the number of tests and the average curd strengths as shown in Columns 3 and 4. There is little evidence of correlation between the curd strength and rennet coagulation time.

The average curd tension of the milk from 31 normal cows (388 tests in all) was found to be 48.56 grams, while the average for 15 infected cows (191 samples in all) was 39.17 grams. The normal herd contained no cows giving milk with an average curd strength below 30 grams, while the infected herd contained 5 cows giving milk with a curd strength of less than 30 grams. The average rennet coagulation time for the normal herd was 7.56 minutes as compared with 10.61 minutes for the infected herd.

A number of the cows with infected udders were milked in such a manner as to keep the milk from the individual quarters separate. Pint samples from each quarter were brought to the laboratory for curd strength and rennet coagulation time measurements. Table 2 presents a summary of the results. Values given in bold face type represent infected quarters,

TABLE 1

Curd strength and rennet coagulation time of milk from normal and infected udders

COW	BREED	CURD STRENGTH		CURD STRENGTH & RENNET COAG.		
		No. of tests	Tension grams	No. of tests	Tension grams	Coag. time minutes
		(NORMAL UDDERS)				
Nell	Holstein	1	48.00	1	48.00	6.12
Girl	"	15	46.40	4	39.75	6.50
Homestead	"	16	36.94	4	36.25	12.43
Becky	"	6	52.17	2	49.00	8.20
Joan	"	15	59.73	4	59.25	6.85
Ida	"	16	63.88	4	63.00	6.38
Rose	"	16	69.00	4	61.00	4.94
Jo	"	16	50.38	5	50.00	13.97
Cordelia	"	13	59.31	2	45.50	14.22
Pearl	"	3	33.67	3	33.67	4.77
Lassie	Ayrshire	16	50.19	3	49.33	12.15
Quince	"	16	50.69	3	53.67	10.52
Priscilla	Guernsey	6	45.17	5	47.40	7.51
Faye	"	16	53.66	4	49.50	9.27
Mabel	"	9	37.22	3	28.33	4.83
Chum	"	9	31.56	1	29.00	6.42
Florence	Brown Swiss	7	35.28	...	.....	.....
Fuschia	" "	6	37.33	3	32.33	5.59
Meridian	" "	8	35.50	4	32.75	7.63
Fairmaid	" "	14	53.50	3	57.00	5.69
Monona	" "	16	31.69	3	28.67	5.08
Fidelity	" "	15	34.47	3	25.67	6.44
Fan	" "	15	34.33	3	37.33	6.50
Eminent	Jersey	15	41.13	2	48.00	5.72
Logas	"	15	54.07	3	51.33	7.54
Safety Belle	"	11	58.18	3	58.67	5.89
Nita	"	17	72.12	5	68.00	6.42
Lotus	"	17	61.94	2	63.00	5.75
Lena	"	16	66.81	2	57.50	8.29
Nina	"	17	70.47	3	69.33	7.67
Fawn	"	10	30.70	3	32.00	7.59
	Average	(388)	48.56	(94)	46.81	7.56
		(INFECTED UDDERS)				
Alice	Holstein	15	27.47	5	30.20	22.36
Anita	"	14	52.78	4	47.50	8.90
Ruth	"	8	39.38	1	36.00	6.73
Inka	"	13	33.07	4	39.50	14.88
Champion	"	12	20.42	2	18.00	17.07
Pontiac	"	11	26.27	2	22.50	5.99
Allee	"	17	26.94	5	26.40	13.22
Lila	Ayrshire	7	38.29	4	36.75	7.63
N. Sally	Guernsey	17	48.59	5	47.00	7.98
C. Sally	"	13	38.00	1	32.00	4.71
Myrtle	"	14	54.86	3	56.33	10.64
Faith	Brown Swiss	15	26.07	4	25.75	13.38
Nanette	Jersey	17	73.53	5	70.40	6.48
Lee Murn	Brown Swiss	17	41.88	5	39.79	10.92
M-85	Holstein	1	40.00	1	40.00	8.26
	Average	(191)	39.17	(51)	37.87	10.61

TABLE 2

Curd strength and rennet coagulation time of milk from individual quarters of infected udders

DATE	COW	BREED	pH BY QUARTERS				CURD STRENGTH BY QUARTERS				COAG. TIME BY QUARTERS				AVE. CURD STRENGTH		AVE. COAG. TIME	
			RF	RR	LR	LF	RF	RR	LR	LF	RF	RR	LR	LF	Normal	Infected	Normal	Infected
2–21	Ruth	Holstein	**6.81***	6.63	6.51	6.50	**23***	38	46	65	**34.68***	9.05	5.63	5.38	49.7	23.0	6.69	34.68
2–21	Inka	"	**6.70**	6.53	**7.15**	6.64	**36**	63	**14**	65	**20.55**	10.03	**108.88**	14.93	64.0	25.0	12.48	64.71
2–21	Champion	"	6.78	**6.82**	**6.79**	6.65	29	**22**	**22**	40	21.90	**28.38**	**21.04**	**9.91**	34.5	22.0	15.40	24.71
3– 1	Pontiac	"	6.39	6.41	**6.95**	6.47	34	31	**2**	35	6.18	7.10	**150++**	6.24	33.3	2.0	6.51	150++
3– 1	Allee	"	6.49	**6.65**	6.49	6.42	27	**12**	27	33	13.70	**39.14**	16.39	11.07	29.0	12.0	13.72	39.14
3–16	Allee	"	6.45	**6.47**	6.36	6.35	19	**8**	26	25	17.68	**39.76**	13.27	11.07	23.3	8.0	14.01	39.76
2–20	Anita	"	**6.63**	6.63	6.61	**6.80**	**45**	42	**41**	**11**	**9.30**	7.59	7.16	**44.53**	41.5	28.0	7.37	26.91
3–15	M-85	"	**6.44**	6.34	6.35	6.35	**26**	35	37	32	**14.56**	13.46	**14.86**	22.69	34.7	26.0	17.00	14.56
2–19	Alice	"	**6.79**	**6.74**	**6.73**	**6.76**	**24**	**27**	**29**	**33**	**37.07**	**24.90**	**26.79**	**23.92**	.....	28.2**	.....	28.17**
2–14	N. Sally	Guernsey	6.51	6.44	**6.87**	6.75	55	61	**13**	**59**	7.74	5.88	**74.29**	6.79	58.3	13.0	6.80	74.29
3–16	N. Sally	"	6.40	6.33	**6.84**	6.39	42	57	**18**	54	10.03	5.99	**91.75**	6.55	51.0	18.0	7.52	91.75
2–15	C. Sally	"	6.40	**6.35**	6.39	**6.24**	52	**10**	53	62	7.34	**3.18**	6.48	**3.79**	52.5	36.0	6.91	3.94
2–20	Lee Murn	Brown Swiss	**6.77**	**6.96**	**6.95**	6.65	26	**10**	**22**	49	15.90	**45.88**	**56.82**	6.61	37.5	16.0	11.25	51.35
2–14	Lila	Ayrshire	6.46	6.48	**6.49**	6.45	56	56	**39**	47	7.89	8.44	**9.54**	7.89	53.0	39.0	8.07	9.54
3–16	Lila	"	6.39	6.40	**6.47**	6.41	55	52	**37**	50	8.56	8.56	**10.46**	8.07	52.3	37.0	8.40	10.46
2–15	Nannette	Jersey	6.39	6.34	**6.36**	6.35	81	83	**68**	76	6.85	6.72	**7.89**	7.15	80.0	68.0	6.91	7.89
3– 9	61A	.....	6.35	**6.73**	6.30	6.30	45	**12**	47	54	6.19	**78.30**	5.51	5.75	48.7	12.0	6.82	78.30
3– 7	3B	.....	6.46	6.44	**6.67**	6.46	28	28	**17**	27	9.24	8.50	**22.51**	9.18	27.7	17.0	8.97	22.51
														Average	45.35	23.65	9.70	43.79

* Bold face figures represent infected quarters.

** Not included in the average because there is no corresponding normal value.

the diagnosis having been made independent of this work as already explained. The average curd strength of the milk from normal quarters was 45.35 grams as compared with 23.65 grams for the milk from infected quarters. The rennet coagulation time was 9.70 minutes for the milk from normal quarters as compared with 43.79 minutes for the infected quarters. The most notable exception from these average indications is presented by the milk from C. Sally; one of the abnormal quarter samples showed a significantly higher curd strength than the normal quarters, and both abnormal quarters showed a decidedly more rapid coagulation with rennet. These results are partly explained by the pH as determined by the quinhydrone electrode. Evidently this cow had an infection which caused the milk to be more acid than normal. This was further confirmed by consulting the records of the veterinarian for January 9th (before this study was started) which showed the following pH values as determined colorimetrically: RF 6.8, RR 5.8, LR 6.7, LF 5.7.

Table 3 gives the results of a similar study of milk samples from individual quarters of cows which had been independently diagnosed as normal. While there are some variations in the curd strength and rennet coagulation of the milks from the four quarters of the same udder, in general these values were far more constant than those obtained with cows having one or more infected quarters as in Table 2. It is interesting to note that the average values obtained for normal cows when the milks were studied by individual quarters agreed quite closely with the average values obtained for normal cows (Table 1) when the entire milking was sampled.

CONCLUSION

Subclinical mastitis causes the milk to have a lower curd strength and to coagulate more slowly with rennet. This result was obtained both when entire milkings from normal and infected udders were compared as in Table 1, and when samples from infected quarters were compared with the milk from uninfected quarters of the same udder. This conclusion is further corroborated by the relatively greater constancy in the curd strength and rennet coagulation of milk from the four quarters of uninfected udders.

Acknowledgment is gratefully made of the cooperation by Professor F. B. Hadley and Professor E. G. Hastings in making their diagnoses and records available to us.

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TABLE 3

Curd strength and rennet coagulation time of milk from individual quarters of normal udders

DATE	COW & BREED	pH BY QUARTERS				CURD STRENGTH				RENNET COAGULATION TIME (MIN.)				AVE. CURD STRENGTH	AVE. RENNET COAG.
		RF	RR	LR	LF	RF	RR	LR	LF	RF	RR	LR	LF		
3- 6	Ida (H)	6.47	6.43	6.43	6.42	65	66	61	58	8.56	7.28	7.40	9.73	62.50	8.24
3-16	M-78 (H)	6.25	6.24	6.25	6.25	30	33	36	37	9.30	9.36	7.77	9.24	34.00	8.92
2-26	Cordelia (H)	6.48	6.40	6.47	6.50	61	54	46	52	14.68	12.11	13.46	14.07	53.25	13.58
3- 1	Jo (H)	6.36	6.39	6.37	6.34	53	47	53	52	12.90	17.30	16.27	12.60	51.25	14.77
2-27	Rose (H)	6.29	6.26	6.25	6.27	62	54	53	72	4.95	4.34	4.52	5.08	60.25	4.72
3- 6	Joan (H)	6.57	6.42	6.60	6.48	54	50	46	64	11.87	8.99	15.54	7.89	53.50	11.07
3-13	Nell (H)	6.30	6.34	6.30	6.30	38	24	37	37	4.77	10.40	4.47	4.59	34.00	6.06
2-26	Quince (A)	6.67	6.51	6.53	6.75	42	61	47	46	14.25	7.83	9.24	13.70	49.00	11.26
3-14	Fan (BS)	6.34	6.36	6.36	6.35	59	54	48	50	6.79	6.48	6.67	6.79	52.75	6.68
3-14	Fidelity (BS)	6.38	6.39	6.37	6.35	39	33	38	32	6.91	6.97	6.61	6.19	35.50	6.67
3- 5	Monona (BS)	6.44	6.37	6.38	6.41	31	36	38	34	5.14	5.01	4.95	5.51	34.75	5.15
3- 6	Fairmaid (BS)	6.36	6.50	6.45	6.35	46	34	44	54	5.93	10.15	5.86	5.81	44.50	6.94
3- 5	Fuschia (BS)	6.36	6.39	6.45	6.50	35	36	34	32	5.57	5.87	5.57	5.62	34.25	5.66
3-13	Florence (BS)	6.30	6.30	6.30	6.34	40	41	42	42	4.89	4.59	4.71	4.65	41.25	4.71
3-14	Chum (G)	6.32	6.30	6.30	6.34	30	36	34	23	6.73	6.36	6.42	6.97	30.75	6.62
3-14	Mabel (G)	6.32	6.30	6.31	6.32	38	44	46	46	5.87	5.57	5.57	5.63	43.50	5.66
3-13	Fawn (G)	6.35	6.35	6.34	6.34	38	42	36	34	8.26	7.83	7.89	8.14	37.50	8.03
3- 1	Faye (G)	6.41	6.45	6.45	6.42	60	61	58	55	10.34	11.38	11.26	10.46	58.50	10.86
2-15	Priscilla (G)	6.38	6.40	6.43	6.40	50	46	36	46	7.58	7.83	8.32	7.10	44.50	7.71
3-16	Enid (J)	6.35	6.35	6.30	6.27	52	51	55	49	5.63	5.02	4.65	4.59	51.75	4.97
3- 5	Nina (J)	6.41	6.40	6.46	6.41	81	75	59	69	7.34	8.32	7.46	7.34	71.00	7.62
3- 5	Lena (J)	6.23	6.24	6.24	6.24	66	66	68	66	6.79	6.97	7.28	7.34	66.50	7.10
2-27	Lotus (J)	6.29	6.28	6.25	6.29	90	92	90	70	6.30	6.18	5.99	6.42	85.50	6.22
2-20	Nita (J)	6.48	6.47	6.54	6.54	84	87	67	70	5.69	5.50	7.77	7.59	77.00	6.64
3-13	Loretta (J)	6.34	6.30	6.32	6.37	59	50	56	49	5.32	5.26	5.08	5.44	53.50	5.27
2-27	S. Belle (J)	6.42	6.40	6.42	6.42	73	72	71	76	7.82	7.03	7.58	7.21	73.00	7.41
2-27	Eminent (J)	6.37	6.34	6.35	6.34	56	61	47	54	5.44	4.95	5.20	5.13	54.50	5.18
3-12	21B	6.33	6.35	6.30	6.30	32	32	35	31	5.02	5.14	4.28	4.34	32.50	4.69
3-12	19B	6.22	6.26	6.25	6.23	38	33	30	42	5.32	5.87	5.51	5.44	35.75	5.54
3-12	Elnora	6.35	6.32	6.40	6.31	53	49	45	54	7.52	6.55	7.89	6.60	50.25	7.14
3-12	16B	6.37	6.44	6.41	6.41	47	48	52	47	6.79	7.16	6.54	7.16	48.50	6.91
3- 8	32A	6.38	6.40	6.40	6.38	53	56	58	59	4.77	6.85	6.55	5.69	56.50	5.97
3- 9	8	6.22	6.32	6.35	6.34	60	60	60	61	6.61	6.42	6.73	6.48	60.25	6.56
3- 9	63A	6.35	6.34	6.35	6.40	49	62	62	49	9.24	10.40	12.79	13.40	55.50	11.50
3- 9	58A	6.28	6.24	6.25	6.34	67	76	64	71	6.85	6.61	7.10	7.30	69.50	6.97
3- 8	13B	6.23	6.25	6.27	6.26	42	43	36	41	4.40	4.65	5.08	4.71	40.50	4.71
3- 7	1B	6.28	6.29	6.27	6.28	47	43	44	50	4.59	4.65	4.40	4.40	46.00	4.51
3- 7	25B	6.31	6.30	6.32	6.32	27	27	27	28	6.42	6.12	6.86	7.30	27.25	6.67
3- 7	10B	6.38	6.37	6.29	6.45	46	40	39	36	6.79	6.91	7.16	9.24	40.25	7.52
													Average	50.03	7.24

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THE STANDARDIZATION OF THE BORDEN BODY FLOW METER FOR DETERMINING THE APPARENT VISCOSITY OF CREAM

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INTRODUCTION

It is a well known fact, that, in spite of constancy of method of cream handling and processing, there is considerable daily and seasonal variation in its viscosity. Due to demand from production men of milk plants for a simple method of estimating cream body, a small simple efflux instrument was developed by Nair and Mook (1). A detailed description and illustrations of the Borden Body Flow Meter is given by them.

Since the Borden Body Flow Meter is coming into use in milk plants for determining the apparent viscosity of cream, it seemed desirable to compare the results obtained with it with results obtained by using a standard instrument such as the MacMichael viscometer. Although the MacMichael viscometer does not give true viscosity for mixtures of colloids and an emulsoid as found in milk and cream, it does give more accurate results than flow types of instruments with changing head pressure.

EXPERIMENTAL

Solutions of sucrose ranging from 20 to 65 per cent were prepared with commercial cane sugar and distilled water. The percentage of sugar in the solutions was determined with saccharometers.

The viscosities of these sucrose solutions were determined in duplicate by the use of the MacMichael viscometer and the Borden Body Flow Meter. The operation of the MacMichael viscometer was in accordance with the recommendations in Eimer and Amend's Bulletin No. 280 using a number 30 wire calibrated with oils of known viscosity. The measurements with the MacMichael viscometer were taken after the oscillation of the bob became approximately constant. The operation of the Borden Body Flow Meter was in accordance with the recommendations of Nair and Mook (1). They also recommended the use of two interchangeable tips; one of 2.78 mm. diameter for cream containing 30 per cent of fat or more and one of approximately 1.97 mm. for thinner creams. Arbitrary temperatures for commercial purposes of 15.6° C. (60° F.) for heavy creams and 10° C. (50° F.) for light creams were chosen by them. The 2.78 mm. tip was used for all

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the work reported in this article. The viscosity determinations of the sucrose solutions were made at 20° C. (68° F.) and those of the cream were made at 15.6° C. (60° F.). The samples were tempered in a water bath in a room held at the temperature at which the determinations were made. Water in the bath of the MacMichael viscometer was also held at the temperature of the determination.

Viscosity determinations were made on two complete series of sucrose solutions. The results secured by the MacMichael viscometer and Borden Body Flow Meter are given in table 1 and the viscosities in centipoises as

TABLE 1

Viscosity of sucrose solutions as determined by the MacMichael viscometer and Borden Body Flow Meter at 20° C. (68° F.)

SUCROSE SOLUTIONS	VISCOSITY WITH MACMICHAEL VISCOMETER	VISCOSITY WITH BORDEN FLOW METER
per cent	*centipoises*	*seconds*
	First series of sucrose solutions	
21.1	3.0	28.0
39.5	7.0	40.0
W*	20.0	87.0
X*	31.0	119.5
61.4	73.5	279.0
63.5	109.0	412.0
64.4	128.8	492.6
	Second series of sucrose solutions	
20.5	3.0	28.0
41.0	7.5	41.0
50.3	16.2	69.5
59.5	52.0	201.5
63.0	95.0	353.5
64.5	129.5	488.0
65.0	168.0	624.5

* The percentage of sugar was not determined.

determined by the MacMichael viscometer are plotted against the seconds as determined by the Borden Body Flow Meter in Figure 1. Figure 1 gives a straight line relationship passing through zero which shows that the Borden Body Flow Meter gave correct results throughout the entire range for a true solution. The points indicating the seconds flow for the less viscous sucrose solutions are slightly below the line probably because the bore of the tip was too large to give accurate results with such solutions. The Borden Body Flow Meter, as previously mentioned, has a tip with a smaller bore to use with thinner liquids, but the results are only slightly in error with the large bore.

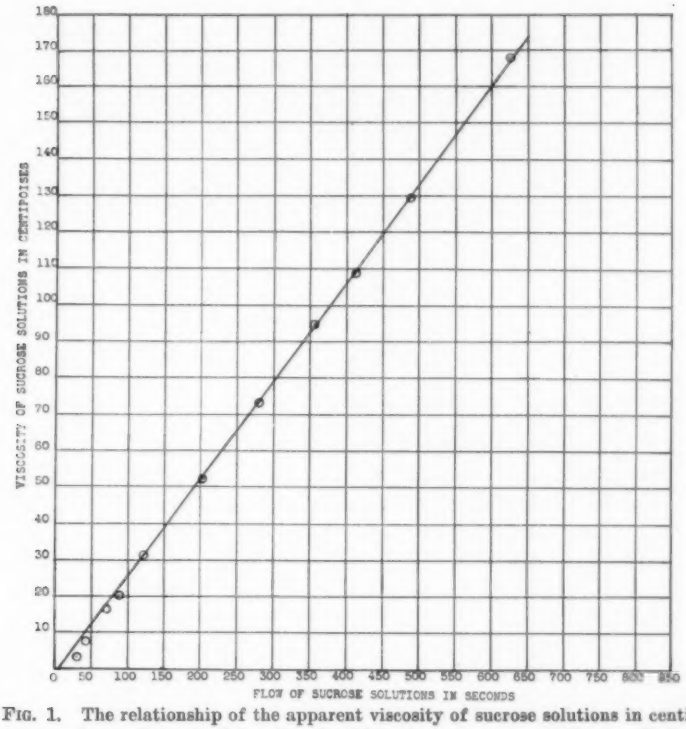

FIG. 1. The relationship of the apparent viscosity of sucrose solutions in centipoises and in seconds as determined by the MacMichael viscometer and Borden Body Flow Meter at 20° C. (68° F.).

The apparent viscosities of creams were determined with the MacMichael viscometer and Borden Body Flow Meter. The pasteurized milk from the station Jersey herd was separated at 61° C. (142° F.) and then the cream was heated to 74° C. (165° F.) and held for 10 minutes before cooling to 10° C. (50° F.) to reduce variations in viscosities due to fat clumping. This cream was standardized to the range of 30.0 to 52.5 per cent of fat, held for 20 hours at 4° C. (39.2° F.), then placed in ½-pint bottles and tempered in a water bath to 15.6° C. (60° F.). All of the creams were held at this temperature during the time required for making the determinations. Tests were made at 2-hour intervals on duplicates of one of the samples to note any change in viscosity due to the holding period. The change in viscosity of the sample appeared to be within experimental error.

The averages of the results of 3 different lots of 30.0, 35.0, and 40.0 per cent cream, 2 different lots of 43.3, 45.0, 47.5, and 50.0 per cent cream, and from 1 lot of 42.0 and 52.5 per cent cream are given in Table 2. The

TABLE 2

Viscosity of cream ranging from 30.0 to 52.5 per cent fat as determined by the MacMichael viscometer and Borden Body Flow Meter at 15.6° C. (60° F.)

AVERAGED NO. TRIALS	CREAM	VISCOSITY WITH MAC MICHAEL VISCOMETER	VISCOSITY WITH BORDEN FLOW METER	
	per cent fat	*centipoises*	*Seconds*	*Centipoises**
3	30.0	12.4	53.0	13.5
3	35.0	16.7	71.5	18.0
3	40.0	25.2	106.5	27.5
1	42.0	32.0	136.0	35.0
2	43.3	34.5	152.5	40.0
2	45.0	38.8	179.7	47.0
2	47.5	51.0	252.0	66.5
2	50.0	74.0	422.0	112.0
1	52.5	102.5	536.0	142.5

* Seconds converted to centipoises by use of Fig. 1 based upon sucrose solutions.

viscosities in seconds of these creams obtained by the Borden Body Flow Meter were transposed to centipoises by means of Fig. 1 based upon standardization of the instrument with sucrose solutions. As can be seen from Table 2, the seconds of flow obtained by the Borden Body Flow Meter when converted to centipoises gave nearly the same results as the viscosity in centipoises as determined by the MacMichael viscometer for creams testing from 30.0 to 40 to 42 per cent fat. For creams of higher fat content there is a marked divergence in the viscosity in centipoises as determined by the Borden Body Flow Meter as compared with the MacMichael viscometer and this divergence increases with each succeeding increase in fat content. The Borden Flow Meter gave too high results which indicated that the results secured on sucrose solutions ought not be transposed to cream.

The relationship of the apparent viscosities of the cream in centipoises, omitting the 52 per cent cream, as determined by the MacMichael viscosimeter and the seconds as determined by the Borden Body Flow Meter are shown in Fig. 2. The results in seconds obtained with the Borden Body Flow Meter with cream show practically a straight line relationship with the results obtained with the MacMichael up to 25 centipoises, but thereafter the relationship is shown by a curved line.

A comparison of the two instruments was made on another series of creams ranging from 30 to 46 per cent milk fat which was separated from milk pasteurized in the usual way and not heated subsequently to a higher temperature. In this case some of the 35.0 and 40.5 per cent cream was treated by the method of Hening and Dahlberg (2) for the purpose of increasing its viscosity. The difference in viscosity due to this treatment was readily detected even at 15.6° C. (60° F.). Nair also reports from unpublished tests that differences in viscosity between heat-treated and non-

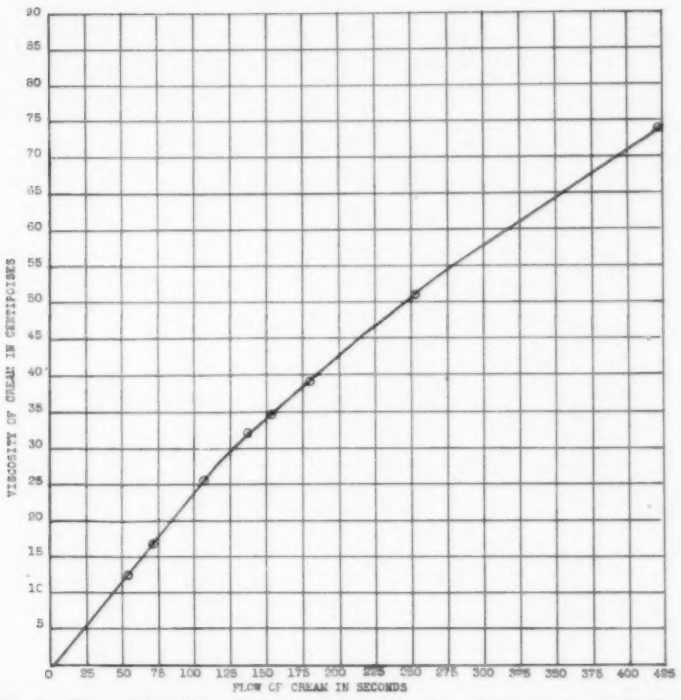

FIG. 2. The relationship of the apparent viscosity of creams in centipoises and in seconds as determined by the MacMichael viscometer and Borden Body Flow Meter at 15.6° C. (60° F.).

heat-treated creams are still demonstrable at a temperature of 15.6° C. (60° F.) using the flow meter. The results obtained with this series of cream were similar to those of the preceding series except that the viscosities of these creams were a little higher because the cream had not been heated to such a high temperature as in the previous tests. The results of these tests are given in Table 3.

As can be seen from Figures 1 and 2 and Tables 2 and 3, if the cream viscosity in seconds for 30, 35, and 40 per cent creams with the Borden Body Flow Meter was transposed to centipoises by means of Figure 1 based upon sucrose solutions, the reading would be 1 to 3 centipoises higher than that determined by the MacMichael viscometer. For creams of higher fat content from 42 to 50 per cent fat the difference would range from 3 to 38 centipoises too high. The difference in the determination with this instrument rapidly increases for creams having fat contents above 42 per cent. However, the flow in seconds of cream with the Bordon Body Flow Meter can be transposed to centipoises by the use of Figure 2 based upon

TABLE 3

Viscosity of cream ranging from 30 to 46 per cent fat as determined by the MacMichael viscometer and Borden Body Flow Meter at 15.6° C. (60° F.)

CREAM	VISCOSITY WITH MAC MICHAEL VISCOMETER	VISCOSITY WITH BORDEN FLOW METER	
per cent fat	*centipoises*	*seconds*	*centipoises**
30.0	13.0	52.0	13.5
35.0	18.0	74.5	19.0
35.0 treated	22.0	87.5	22.5
40.5	30.0	126.0	33.0
40.5 treated	46.5	215.0	56.8
46.0	60.5	303.0	80.5

* Seconds converted to centipoises by the use of Fig. 1 based upon sucrose solutions.

results secured with cream. It should be possible by the use of Figure 2 based upon the standardization of the Flow Meter with cream to transpose the viscosity of cream in seconds to centipoises, the standard unit for expressing viscosity. In fact, if the seconds obtained with the Borden Body Flow Meter for the series of cream in Table 3 are converted to centipoises from Figure 2, the results check very close with those obtained with the MacMichael viscometer.

DISCUSSION AND CONCLUSIONS

In these experiments it has been shown that the Borden Body Flow Meter gave consistent results on the same and on different samples of cream. The accuracy of the apparatus was equally good on creams containing from 20 to 40 per cent of fat using only the large-bore tip of 2.78 mm. diameter. The same tip and temperature appeared satisfactory for the range of fat contents of market creams.

Standardization of the Borden Body Flow Meter was readily accomplished with sugar solutions and the seconds of flow showed a straight line relationship to the viscosity in centipoises. These results applied to the apparent viscosities of creams only up to 25 centipoises (about 100 seconds) which is the approximate viscosity of 35 to 40 per cent cream at 15.6° C. (60° F.). For creams of higher viscosity the Borden Body Flow Meter consistently gave results which were too high. However, results on very viscous creams may be transposed to approximate centipoises by the standardization data secured on cream, thereby making it possible for those using this Flow Meter to compare their results quite accurately with data reported in centipoises.

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THE AGE THICKENING OF SWEETENED CONDENSED MILK

I. SEASONAL VARIATIONS*

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Manufacturers of sweetened condensed milk have frequently found that during storage their product would undergo a thickening to the extent that it no longer remained a syrupy liquid but had the consistency of a custard. In some cases the thickening may have been due to the growth of certain types of bacteria which represented a spoilage of the product, while in other cases the thickening was due to physical or chemical changes which took place on aging but which was not accompanied by any spoilage of the product. Bacterial thickening may be prevented by having the sucrose concentration high enough to inhibit the growth of bacteria. Colloidal or age thickening has been found to be most prevalent during the late spring and early summer. Even though age thickening is not accompanied by the spoilage of the product, such a heavy body is objectionable from the standpoint of the consumer. The factors involved in this type of thickening have not been well understood and manufacturers of this product have refrained from canning the milk for retail trade during the period of the year when the milk was unstable.

REVIEW OF THE LITERATURE

Effect of Storage Temperature:—Rogers, Deysher and Evans (1) found that sweetened condensed milk thickened more rapidly at high storage temperatures, but that the increase in viscosity was not in direct relation to the temperature. They reported no increase in viscosity when the milk was stored at 10° C. (50° F.) for 121 days, a slight increase at 20° C. (68° F.), and a marked increase in viscosity at 30° C. (86° F.) and higher.

Miyawaki (2) states, "In general, it may be said that about three weeks of incubation corresponds to about six months of aging under natural conditions." However, he does not state the temperature of incubation.

Leighton and Mudge (3) determined the length of time for thickening to begin in condensed milk stored at various temperatures and concluded that the reaction at the higher and lower temperatures was identical. These investigators have also shown that the heat coagulation of the casein of milk is an endothermic reaction and that the heat absorption comes from the precipitation of calcium and magnesium as phosphates and citrates. The

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heat absorption obtained with condensed milk was greater than that obtained from a serum made up according to the formula of Van Slyke and Bosworth. If enough extra calcium was added to make up for the amount combined with the protein the heat absorption of the serum was commensurate with that obtained with condensed milk.

Effect of Forewarming Temperature:—Hunziker (4) found that forewarming temperatures up to 167° F. had a slight tendency toward stabilizing the milk over the unforewarmed milk. From 167° F. to boiling the effect was to unstabilize the milk, while temperatures above boiling increased the stability and diminished the tendency to thicken. He recommends that the forewarming temperature be limited to 167–170° F. for 10 minutes in order to control the thickening.

Leighton and Deysher (5) found that a forewarming temperature of 65° C. (149° F.) slightly stabilized the product as compared to that made from unforewarmed milk; at 75° C. (167° F.) there was also a slight stabilizing effect; at 85° C. (185° F.) a decrease in stability set in, which was very marked at 95° C. (203° F.); and increased stability followed upon forewarming at 110 and 120° C. (231 and 248° F.).

Rogers, Deysher and Evans (1) have shown that milk forewarmed at 80° C. (176° F.) thickened much more rapidly than milk forewarmed at 60° C. (140° F.). They reported that satisfactory results may be obtained by forewarming to about 63° C. (145.4° F.) for 20 to 30 minutes. This temperature reduced the tendency of the milk to thicken during storage but there was danger of fat separation.

Sommer (6) reported that high forewarming temperatures in the case of evaporated milk tended to stabilize the product when subsequently subjected to sterilizing temperatures. Leighton and Deysher (5) and Leighton and Mudge (3) have shown that when stability was plotted against forewarming temperature the curve obtained for sweetened condensed milk was the reverse of the curve obtained for evaporated milk.

According to Leighton and Mudge (3), continued forewarming at 95° C. resulted in a stabilization of the product, but the effect was not as marked as in the highly forewarmed product. However, their stability figures were not consistent with their observations on the viscosity.

Leighton and Deysher (5) concluded that the temperature of forewarming was the major factor in determining the resistance to heat of both evaporated and condensed milk and that the time of forewarming was not so important but could not be neglected.

Effect of Air:—Experiments by Rogers, Deysher and Evans (1) in which samples of sweetened condensed milk were stored in evacuated bulbs, showed that air was not a factor in the age thickening of the milk.

Effect of Homogenization:—Rogers, Deysher and Evans (1) showed that while homogenization of sweetened condensed milk had the effect of increas-

ing the initial viscosity it was without appreciable effect on the progressive increase in viscosity.

Effect of Reaction:—Rogers, Deysher and Evans (1) reported that variations in the acidity within reasonable limits had no appreciable effect on the viscosity of sweetened condensed milk. Batches of milk in which they adjusted the acidity before condensing from pH 6.51 to 6.30, 6.41 and 6.63 showed no real difference in viscosity upon aging. However, in one experiment in which the reaction had been materially changed the milk thickened in the pan. In the above experiments the skimmilk was forewarmed at 63° C. (145.4° F.) and the samples showed practically no increase in viscosity at the end of 30 days' storage at 30° C. (86° F.). Hunziker (4) is of the opinion that had they used a higher forewarming temperature the samples with the higher acidities would have probably shown a decrease in stability toward age thickening.

Leighton and Mudge (3) found that the pH of the artificial serum of Van Slyke and Bosworth changed from pH 6.59 to approximately 6.25 upon precipitation produced by heating, but there was no change in the reaction of sweetened condensed milk upon thickening.

Effect of the Sucrose:—Rogers, Deysher and Evans (1) found that the cane sugar concentration had only a minor influence on the initial viscosity and probably none at all on the progressive increase in viscosity. In these experiments on the effect of sugar concentration a forewarming temperature of only 48° C. (118.4° F.) was used and Hunziker (4) points out that had they used a forewarming temperature high enough to encourage colloidal thickening that probably the sucrose concentration would have had an influence on the subsequent thickening.

Hunziker (4) has found that increasing the amount of sugar tended to minimize the thickening tendency, even in the milk made in early summer. He suggests that probably the higher concentration of sucrose may physically hinder hydration and swelling.

Leighton and Deysher (5) found that sugar exerted its influence on the stability in the forewarming process, in the evaporating process and in the coagulating process. Its effect was to unstabilize the 95° C. forewarmed product and to stabilize the 120° C. material. Based upon the findings of Leighton and Mudge (3) that the heat absorption incident to coagulation comes from the precipitation of calcium and magnesium, Leighton and Deysher (5) believe that the sugar effect has its action upon the metallic salts of milk since cane sugar can react with calcium and since some calcium and magnesium salts dissolve in sugar solutions.

Leighton and Mudge (3) are of the opinion that the sugar accelerates the removal of the calcium from an active state under the conditions of prolonged forewarming.

Ramsey, Tracy and Ruehe (7) found that where dextrose was used to

replace part of the sucrose in sweetened condensed milk that the physical thickening was greatly accelerated, but which could be prevented by adding the corn sugar as a concentrated solution near the end of the run instead of adding it to the milk before forewarming.

Effect of Fat:—Miyawaki (8) has shown that condensed milk made from fat-poor milk does not in general remain syrupy long, but tends to become pasty. He recommends that the raw milk should contain at least 3 per cent fat.

Effect of Casein and Albumin:—Rogers, Deysher and Evans (1) found that the casein and albumin were the milk constituents directly responsible for age thickening, and that casein had, by far, the greatest influence. Albumin became a factor in the thickening only when forewarming temperatures above its coagulation point were used.

Effect of Concentration of Milk Solids:—Rogers, Deysher and Evans (1) have shown that increasing the concentration of the milk solids increased the tendency of the sweetened condensed milk to thicken. The tendency to thicken was not in direct proportion to the concentration of the solids but increased more rapidly at a concentration of 26 to 28 per cent than at lower concentrations.

Effect of Salts:—Rogers, Deysher and Evans (1) found no appreciable effect on the viscosity by adding 2.8 grams of calcium citrate to 3,500 grams of skimmilk when the finished product was held at 30° C. (86° F.) for 58 days. With the addition of enough monobasic phosphate to increase the total phosphates of the milk 5 per cent there was no significant difference in the viscosity authough it was somewhat more viscous than the control batch. The addition of phosphates in amounts to raise the total phosphates by 7 to 11 per cent caused a decided increase in the thickening tendency in 23 days, and much more so in 44 days. These investigators also found that the addition of copper salts failed to show any increase in viscosity over the control.

Effect of Vacuum Pan Temperature:—Hunziker (4) found that finishing the batch at a relatively high temperature (150° F. or above) yielded a product with higher initial viscosity and thickening tendency than finishing at relatively low temperatures (120 to 130° F.).

Miyawaki (2) recommends keeping a vacuum above 26 inches on the pan in order to secure a good keeping quality milk.

Effect of Season of the Year:—As is well known by manufacturers of sweetened condensed milk, and as pointed out by Hunziker (2), the greatest difficulty with rapid thickening occurs during the late spring and early summer.

Sommer (6) has pointed out that the troublesome heat coagulation in the case of evaporated milk is most prevalent during the fall, winter, and early spring.

EXPERIMENTAL METHODS

The Vacuum Pan:—The various batches of sweetened condensed milk used in these experiments were made in an experimental pan devised to approximate commercial conditions as nearly as possible and at the same time permit accurate control as well as the use of a small amount of milk for a trial. The pan is shown in figure 1. Condensing was done in balloon

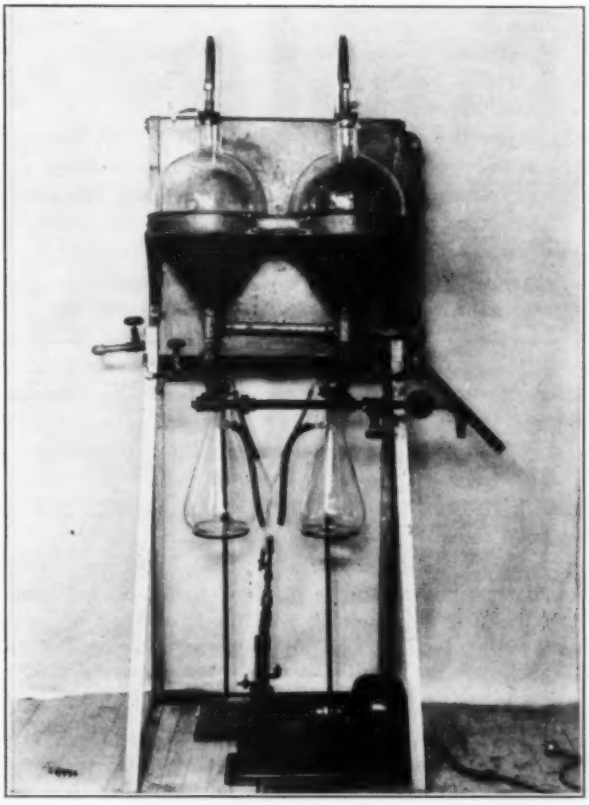

FIG. 1. Experimental vacuum pan

flasks of 12 liters capacity, the heat being applied by means of live steam in a copper-jacket surrounding the lower one-third of the flask. The vacuum was maintained with a motor-driven vacuum pump, attached, through a suction flask, to a copper coil condenser submerged in a tank of running water. The condenser was attached to the condensing flask with

a rubber stopper which also served to hold a thermometer and a glass stopcock used to regulate the flow of milk into the flask. Two such units were made into one piece of apparatus so that a control batch could be run at the same time as a variable factor was being tried on the other batch. The apparatus was also provided with a vacuum gauge. With this arrangement it was possible to control accurately the temperature and vacuum in both flasks.

Milk:—Except in the experiments with the milk of individual cows, mixed milk from the University Creamery was used. About 8 pounds of milk were required for a batch and were standardized to give 3.4 pounds of the finished product containing approximately 8 per cent fat, 20 per cent milk solids not fat, and 44.1 per cent sucrose.

Forewarming:—The forewarming was done in five-gallon "shot gun" cans placed in a water bath heated with steam. In some cases where a high forewarming temperature was desired the steam was introduced directly into the milk. Unless otherwise stated, the sugar was added to the milk before heating was commenced and the milk was forewarmed to 88° C. (190.4° F.) for a period of 10 minutes before drawing it into the pan.

Condensing:—The vacuum was regulated so that condensing was done at a temperature of 55° C. (131° F.). In finishing the batch the vacuum was increased, thus decreasing the temperature of the milk so as to avoid any possibility of superheating. It required about 2½ hours to complete the evaporation. After the condensing was judged complete the flasks were disconnected and weighed on a sales counter scale and distilled water added to make up the contents to the desired weight. After a little experience it was possible to judge the end point so that it was seldom necessary to add more than a small amount of water.

Storage of the Condensed Milk:—In some of the first experiments the milk was stored in tin cans and in one-half pint milk bottles sealed with paraffin. In the later trials, when a different method of measuring the viscosity was adopted, the milk was put up in rubber-stoppered 50 × 400 mm. test tubes. Storage was made at 37° C. (98.6° F.) in order to hasten thickening.

Viscosity Measurements:—In the early trials the viscosity of the condensed milk was measured with the MacMichael viscometer using a number 30 wire, but for various reasons this instrument was not suited to this work. The use of this instrument necessitated the removal of the milk from the container and stirring. Stirring the sample decreased the viscosity which made it difficult to stir all samples alike. An attempt was made to obtain the "basic" viscosity by first forcing the milk through a capillary tube, but this was found to be impractical because the milk began to thicken again as soon as it left the tube. However, what was really desired was not the "basic" viscosity of the milk but its consistency as the consumer would find it upon opening the container.

These objections to the use of the MacMichael viscometer were eliminated by the falling-sphere method of measuring viscosity, using a vacuum tube arrangement to detect when a steel ball passed through two coils of wire placed 22.7 cm. apart. When a viscosity measurement was to be made the tube of milk was inserted through the coils, a steel ball dropped in the top, and its fall timed with a stop-watch while it fell the 22.7 cm. The apparatus was designed to show a deflection on a galvanometer needle when a steel ball passed through the coils, causing a change in the amplitude of oscillations in the oscillating circuit. After making a measurement the steel ball was allowed to remain in the tube until the milk was discarded. This method was very rapid and had the advantage of permitting a viscosity measurement to be made with the minimum of disturbance to the milk. A diagram of the apparatus, including a list of the various parts is shown in figure 2.

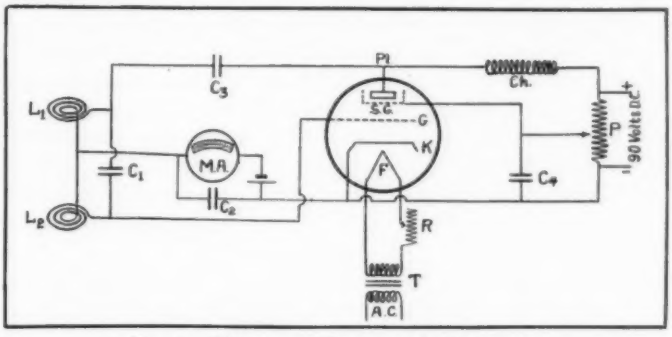

FIG. 2. Diagram of Vacuum Tube Viscometer

L_1, L_2—10 turns each of No. 16 or No. 18 double cotton covered enameled wire.
Ch.—1000 turns of No. 38 enameled wire or R.F. choke for 3000 kilocycles.
C_3—0.0005 microfarad mica condenser. Select one having less than 0.01 milliampere leakage.
C_2, C_4—0.5 microfarad paper condensers.
C_5—0.01 microfarad paper condenser.
M.A.—0 to 1.0 milliampere meter.
P—10,000 ohm, wire wound only, potentiometer of good quality.
R—2.0 ohm rheostat, wire wound only.
T—110 volt A.C. transformer with 2.5 volts output.

Screen grid vacuum tube, type 235.

Pl.—Plate.
S.G.—Screen grid.
G.—Grid.
K.—Cathode.
F.—Filament.

Viscosity measurements were made at 37° C. (98.6° F.), the temperature of storage, and the results calculated to absolute units according to Stokes'

formula. Steel balls of three different diameters were used, namely: 0.6345, 0.9520 and 1.2695 centimeters. Where the viscosity of the samples was so great that it required more than 2 minutes for a steel ball with a diameter of 1.2695 cm. to fall 22.7 cm. through the milk it was reported as being too viscous to measure. When the viscosity was high there was a tendency for the steel ball to slide to the side of the tube rather than to fall straight down.

I. SEASONAL VARIATIONS

The prevention of fat separation in sweetened condensed milk depends upon having the viscosity of the milk great enough to prevent the rise of the fat globules. Normal sweetened condensed milk has a viscosity of about 6 to 8 poises at 37° C. (98.6° F.) shortly after it is dropped from the pan, but a further increase in viscosity during storage is desirable because this initial viscosity is not great enough to prevent fat separation, especially if the milk is held in storage for any great length of time.

Normal sweetened condensed milk will show a gradual increase in visvosity during storage, the rapidity of the thickening depending upon the temperature at which it is held. However, there is a certain period during the year when the milk has the tendency to thicken abnormally fast, especially at room temperature or higher. During this unstable period it is not uncommon to have a sample thicken so rapidly that at the end of 24 hours' storage at 37° C. (98.6° F.) the container may be inverted without loosing its contents.

This unstable period is usually referred to as occurring during the late spring and early summer, especially during May and June. The cause of the unstability during this season of the year is not definitely known, but is no doubt due to some change in the composition of the milk at this time. This being the case we should expect the date of change from stable to unstable milk to vary with the community and from year to year.

The milk used in these trials was taken from the raw milk storage tank of the University Creamery and consisted of the mixed milk from several farms. This milk was fairly representative of the milk from the area of Madison, Wisconsin.

In the spring of 1932 the batch made on April 23 thickened in a normal manner during storage, while that made on April 24 and the subsequent batches thickened abnormally fast. After May 20 no condensing was done until June 24 when the milk was relatively more stable than it was during May and the latter part of April. These results are shown in Table 1.

In 1933 condensing was not begun until April 15 and the milk was already unstable at that date as shown in Table 2. The last batch was condensed on May 27 and the milk was still unstable.

Table 3 shows the progressive increase in viscosity during storage of six batches of sweetened condensed milk made during a period extending

TABLE 1

Seasonal changes in stability during the spring and summer of 1932

DAYS AGING	APRIL 18	APRIL 23	APRIL 24	MAY 2	MAY 9	MAY 14	MAY 20	JUNE 24	JULY 7	JULY 19	JULY 20
					Viscosity in Poises						
0	4.9	6.2	7.4	6.2	4.9	24.3	22.9	7.4	8.7	6.2	7.4
1			230.0				*	112.2	36.2	14.8	18.6
2		44.6		138.5	130.0	*					
3				*							
4	162.3	163.2		422.0	1190.0				273.1		
5								792.2			
6	317.8			1981.0	*						568.0
8									740.0	69.3	
9	293.0	460.0									
10								568.0			
11				2031.0							
12											
13		258.1									
14	374.2			*					773.0	28.4	660.0
16		213.0						525.7			
18	323.0										
20								1088.0			
21	377.6										
25		407.6									
26								795.0			
27									1885.0	1116.0	
30	992.0	505.1									

* Samples too viscous to measure.
Storage temperature 37° C. (98.6° F.).
Forewarming temperature 88° C. (190° F.) for 10 minutes.

TABLE 2

Seasonal change in stability during spring 1933

DAYS	APRIL 15	APRIL 22	APRIL 29	MAY 4	MAY 15	MAY 25	MAY 27
				Viscosity in Poises			
0	16.1	41.0	14.8	18.6	22.3	38.4	44.6
1	144.0	1050.0		584.0		*	
2	865.0	*		2205.0			1850.0
3	*		*	*			
5					*		*

Forewarming temperature 88° C. (180° F.) for 10 minutes
Storage temperature 37° C. (98.6° F.).
* Too viscous to measure.

from April 27, 1934, to May 29, 1934. The batch made on May 15, 1934, had a normal initial viscosity and thickened in a normal manner, while the batch made on May 19, 1934 had a high initial viscosity and thickened

TABLE 3

Seasonal change in stability during spring 1934

DATE OF CONDENSING	APRIL 27	MAY 8	MAY 15	MAY 19	MAY 24	MAY 29
Sucrose	44.1	44.1	44.1	44.1	44.1	44.1%
Fat	7.55	8.29	7.52	8.31	8.05	7.16%
Milk solids not fat	20.67	20.73	20.83	21.43	20.80	20.34%
Days			Viscosity in Poises			
0	7.4	7.4	6.2	31.0	17.3	38.4
1		25.6	14.8		1038.0	1488.0
2				*		*
3	100.4	125.0				
4			58.5	Solid	*	
5	131.3					
7		135.0				
8			82.4			
10	529.0					
11		158.9				
13			95.0			
14	470.7					
17		204.1				
18	539.8					
20		178.7	124.1			
22	740.1					

Forewarming temperature
Storage temperature 85° C (185° F) for 10 minutes. 37° C (98.6° F).
* Too viscous to measure.

abnormally fast. In 1934 the unstable period did not commence until approximately a month later than in 1933 and 1932.

Trials could not be carried out during the summer of 1933 and 1934, so it is not known when the unstable period ended. The data given in Table 1 tend to show that the change again from unstable milk is not as abrupt as is the change from stable to unstable milk but occurs during the latter part of June and the first part of July.

That the change from stable to unstable milk is very abrupt is shown in Table 1 where the milk condensed on April 23, 1932, was stable while that condensed on April 24 was very unstable. Table 3 shows that during the spring of 1934 the change occurred between May 15 and May 19. If batches had been condensed during the intervening 3 days it might have been found that the change occurred over night.

DISCUSSION

It is impossible to state any definite limit of viscosity above which the milk may be considered as unstable and below which the milk may be said to be stable. However, numerous trials have shown that when unstable milk was stored at 37° C. (98.6° F.) the thickening was usually so rapid that it attained a viscosity of over 3000 poises (too viscous to measure) within a few days after manufacture, while the viscosity of stable milk under the same conditions of storage was usually below 1000 poises at the end of 3 weeks. The initial viscosity of stable sweetened condensed milk averaged from 6 to 8 poises, while unstable milk was usually somewhat higher. However, it was not always possible to predict the rate of thickening of the milk from the initial viscosity.

Hunziker (4) suggests that probably the cause of the unstable period during the spring is due to a large majority of the cows freshening at that time thus causing a high albumin content in the milk. This explanation would not hold for this territory where most of the cows freshen earlier in the year and at best would not explain the abrupt change to unstable milk. The milk at this season of the year is no doubt relatively high in albumin and it may be a factor.

It has been observed in the evaporated milk industry that the change from unstable to stable milk in the spring also occurs very abruptly but this has been explained as being due to the increased citrate content in the milk caused by green pasture feed. Unstable sweetened condensed milk cannot be attributed entirely to the change in the milk occasioned by green feed, but it may be a factor. The change to green feed would not explain the fact that the unstable period in 1934 did not occur until approximately a month later than in the two previous years. The spring of 1934 was relatively hot and dry. During the latter part of June and July in 1932 the milk was much more stable than it was during May even

though the cows were still getting plenty of good green feed. In some trials where the milk of individual cows was condensed it was found that some cows (especially Jerseys) gave milk that was consistently unstable even though they were on winter rations.

CONCLUSIONS

The unstable period for sweetened condensed milk begins very abruptly and may occur any time from the middle of April to the middle of May in the territory of Madison, Wisconsin.

The change back from unstable to stable milk appears to occur more slowly and takes place during the latter part of June and July.

The freshening of the cows or the time at which they are turned out on grass cannot be correlated directly with the period during which sweetened condensed milk is unstable toward age thickening.

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THE DIKETONE PRODUCED WHEN BUTTER CULTURES ARE STEAM DISTILLED WITH FERRIC CHLORIDE ADDED*

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The relationship of diacetyl to the desirable flavor of butter has been established by the work of various investigators. When a satisfactory butter culture is steam distilled and the distillate treated with suitable reagents, a brick red precipitate is formed that is commonly considered to be nickel dimethylglyoximate, a derivative of diacetyl. Much more precipitate is formed if ferric chloride is added to the butter culture before distillation, and this is interpreted as indicating the formation of relatively large amounts of diacetyl through an oxidation of acetylmethylcarbinol present in the culture.

Since certain homologues of diacetyl have been assumed to be valuable for the purpose of giving oleomargarine a desirable flavor and aroma (3) and also because these homologues are similar to diacetyl from various standpoints, it seemed advisable to determine whether or not the diketone obtained on distilling a butter culture with ferric chloride is diacetyl. The general procedure followed was to prepare the usual nickel salts from the distillates of butter cultures and then determine the nickel contents for comparison with the theoretical nickel content of nickel dimethylglyoximate.

METHODS

A relatively large amount of each butter culture was steam distilled, after adding ferric chloride, and the distillate treated with hydroxylamine hydrochloride, sodium acetate, and nickel chloride solutions; 200 ml. of 40 per cent ferric chloride solution (40 gm. made up to 100 ml. with distilled water) were used per liter of butter culture, and the other reagents were employed in amounts sufficient to insure complete precipitation of the diketone present. Each precipitate was washed repeatedly with distilled water by decantation and then collected, with suction, on a crucible having a fused-in sintered filter disc; the precipitate was also thoroughly washed on the crucible. The salt was removed from the crucible and dried at slightly more than 100° C. During the decantation there was some loss of the precipitate, so the salt was determined quantitatively on a 200-gm. portion of each butter culture, using the procedure followed by Michaelian

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and Hammer (2) for determining both the diketone present originally and that resulting from an oxidation with ferric chloride.

The nickel content of each salt was determined by decomposing, with sulfuric and nitric acids, a portion that had been thoroughly dried and precipitating the nickel as dimethylglyoximate, which was collected and weighed.

RESULTS OBTAINED

The results obtained are given in Table 1. The percentages of nickel in the various salts prepared from butter cultures ranged from 20.14 to 20.30, all of the values being close to the theoretical for nickel dimethyl-

TABLE 1

Nickel content of salts obtained when butter cultures were steam distilled with ferric chloride and the distillates treated with hydroxylamine hydrochloride, sodium acetate and nickel chloride

Butter cultures developed at 21° C.

BUTTER CULTURE			GM. OF PURIFIED NI SALT OBTAINED	% NI IN NI SALT	MG. NI SALT OBTAINED QUANTITATIVELY FROM 200 GM. CULTURE
No.	General quality	Ml. distilled			
15	excellent	3,000	0.5558	20.24, 20.20 } 20.22	45.8
146	fair	2,750	0.3524	20.29, 20.31 } 20.30	33.2
15	good	8,500	1.1838	20.25, 20.20 } 20.225	39.6
232	good	10,000	1.7534	20.17, 20.11 } 20.14	43.5
15*	**	10,000	3.2555	20.22, 20.25 } 20.235	84.1

* 0.15% citric acid crystals added to milk from which butter culture was made.

** Flavor and aroma were typical of a butter culture made from milk to which citric acid had been added.

glyoximate[1] and distinctly higher than the theoretical values for the higher homologues. An average nickel content of 20.30 per cent was obtained on the nickel salt made from a commercial preparation of diacetyl. When citric acid was added to the milk used in preparing the butter culture, the salt obtained had the same nickel content as the salts obtained when no citric acid was added to the milk. The amounts of nickel salt obtained quantitatively per 200 gm. of the various cultures were normal for butter cultures of a satisfactory quality (1).

[1] The theoretical Ni content of nickel dimethylglyoximate = 20.32 per cent; of nickel ethylmethylglyoximate = 18.52 per cent; of nickel diethylglyoximate = 17.02 per cent.

From the data presented it appears that the diketone steam distilled from butter cultures after the addition of ferric chloride is diacetyl rather than one of the homologues and that if homologues are present they are limited to relatively non-significant amounts.

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(3) U. S. Patent 1,945,347.

MOLDINESS IN ROMANO CHEESE*

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INTRODUCTION

A factory manufacturing Cacio Pecorino Romano cheese, more commonly known as Romano cheese, from cows' milk reported that they were having considerable trouble with mold developing in the center of many of the cheeses. Inasmuch as this was undesirable in this type of cheese, they were anxious to find the cause and remedy the condition. Several cheeses in which the mold was present in the center and several cheeses in which no mold was present were submitted for study and analysis.

In making Romano cheese the procedure usually followed is to soak the cheese in a 30° salometer brine for about 3 days. After this the cheese is removed from the brine and salt is rubbed into it every day for four days. The cheese maker at this particular factory had punched the cheese full of holes to allow greater penetration of the brine. After the cheese had been removed from the brine and stored for aging, the holes permitted the air as well as mold spores to enter the cheese. In all cases the moisture content was sufficient to permit the growth of mold. Doane and Lawson[1] and also Matheson[2] in the revised edition of U. S. D. A. Bulletin 608, p. 36, in describing Romano cheese and its manufacture, referring to salting, state: "This process is sometimes facilitated by punching several holes in the cheese." In the light of the present work, it is inadvisable to follow this procedure.

METHOD OF ANALYSIS

Samples of the moldy cheese were ground in a sterile mortar with sterile sand and plated on malt extract agar. A pure culture of mold was isolated and identified as *Penicillium italicum*.

In order to study the conditions which favored the development of this mold in the cheese, analyses were made of the moisture and sodium chloride content of cheese that had molded and of cheese that had not molded. The moisture content was determined by weighing approximately 10 grams of the cheese in a platinum crucible and drying in a vacuum oven at 56° C.

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* Journal Article No. 222 (n.s.) from the Michigan Agricultural Experiment Station.

[1] Doane, C. F., and Lawson, H. W. Varieties of cheese: descriptions and analyses. U. S. D. A., Bul. 608, (1918).

[2] Doane, C. F., Lawson, H. W., and Matheson, K. J. Varieties of cheese: descriptions and analyses. U. S. D. A., Bul. 608, (1928).

TABLE 1

Diagram of section of cheese in which no mold was present showing the percentage of moisture and sodium chloride present

1 H_2O = 12.0 NaCl = 7.4	2	3	4	5	6	7 H_2O = 13.0 NaCl = 7.5
8	9 H_2O = 15.0 NaCl = 7.7	10 H_2O = 18.0 NaCl = 7.6	11 H_2O = 21.0 NaCl = 7.5	12 H_2O = 15.0 NaCl = 7.8	13 H_2O = 16.0 NaCl = 7.9	14
15	16 H_2O = 21.0 NaCl = 7.8	17 H_2O = 15.0 NaCl = 8.0	18 H_2O = 17.0 NaCl = 7.8	19 H_2O = 16.0 NaCl = 7.8	20 H_2O = 17.0 NaCl = 7.6	21
22 H_2O = 17.0 NaCl = 7.3	23	24	25 H_2O = 16.0 NaCl = 7.8	26	27	28 H_2O = 20.0 NaCl = 7.5

until the weight was constant. The sodium chloride content was determined by igniting one gram of cheese in a platinum crucible. The resulting ash was then dissolved in 10 cc. of water and titrated with n/10 $AgNO_3$, using a one per cent K_2CrO_7 solution as the indicator.

In order to study the relationship between the moisture and sodium chloride content and moldiness in the various samples of cheeses, the cheese was cut into slices about one inch thick and these were diced and numbered. The results of typical analyses are given in the following diagrams representing the slices of the diced cheese.

TABLE 2

Diagram of section of moldy cheese showing relation between the percentage of moisture and sodium chloride and the presence of mold in the cheese. Mold confined to section 8

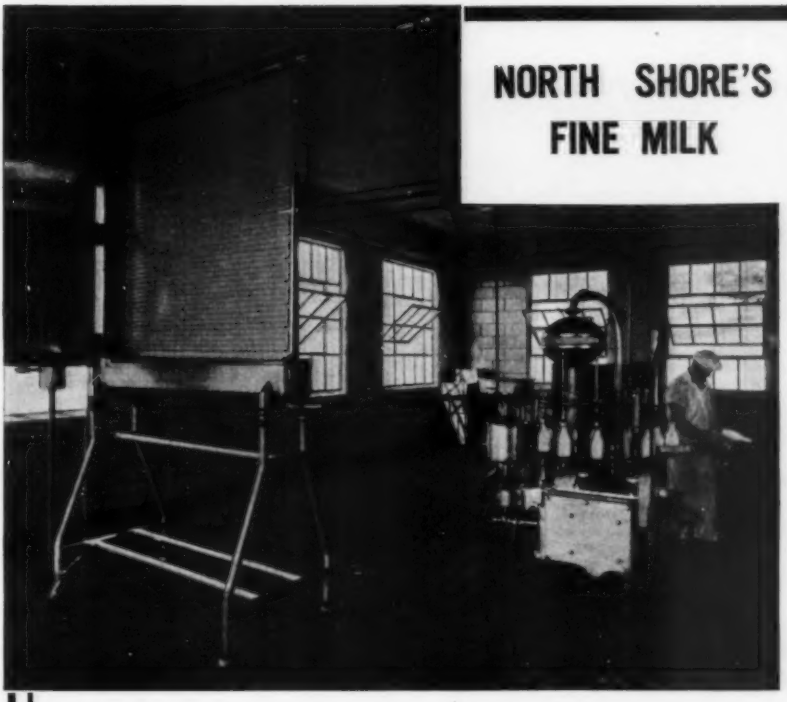
1 H_2O = 15.7 NaCl = 7.5	2 H_2O = 18.4 NaCl = 7.5	3 H_2O = 18.0 NaCl = 7.0	4 H_2O = 19.9 NaCl = 6.7	5 H_2O = 11.1 NaCl = 7.5
6	7	8 H_2O = 24.5 NaCl = 6.0	9	10
11	12 H_2O = 20.1 NaCl = 5.8	13 H_2O = 19.8 NaCl = 7.2	14 H_2O = 23.9 NaCl = 6.3	15
16 H_2O = 17.7 NaCl = 7.4	17	18 H_2O = 18.6 NaCl = 7.5	19	20 H_2O = 16.1 NaCl = 8.2

INOCULATING CHEESE WITH MOLD

Squares of cheese from a cheese which showed no mold were placed in deep culture dishes. Mold spores from the mold isolated from the moldy cheese were then spread on the surface of the cheese and incubated a week with no growth resulting. One cc. of water was then added to the squares, but no growth resulted. It was necessary to repeat this twice before the mold grew on the surface of the cheese.

Our results would indicate that the moisture content of the cheese would have to be approximately 25 per cent and the salt content not greater than 6.0 per cent to permit mold growth to take place. Other sections of the cheese having a greater salt content or a smaller amount of moisture were not affected. In the cheese where no mold growth occurred, the moisture content was less than 25 per cent and the salt content greater than 6 per cent.

These results most certainly indicate that in making Romano cheese, it is not advisable to punch holes in the cheese to facilitate the penetration of the brine into the interior. This may permit molds to grow in the center. Molds developed in every cheese in this factory so punctured.



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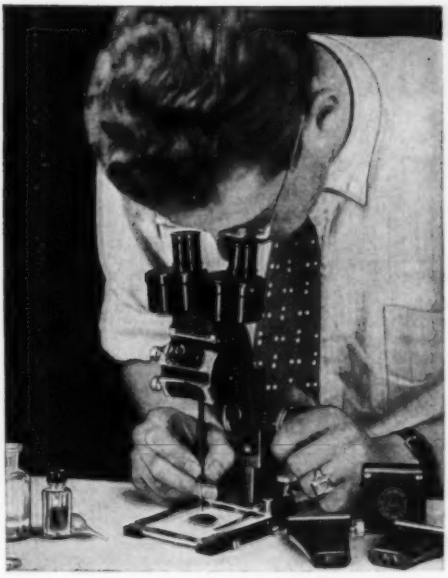

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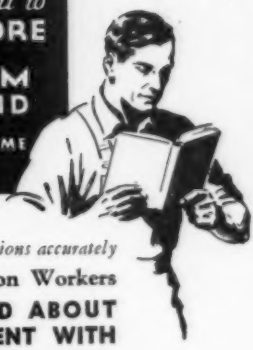

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